New Material Proposal

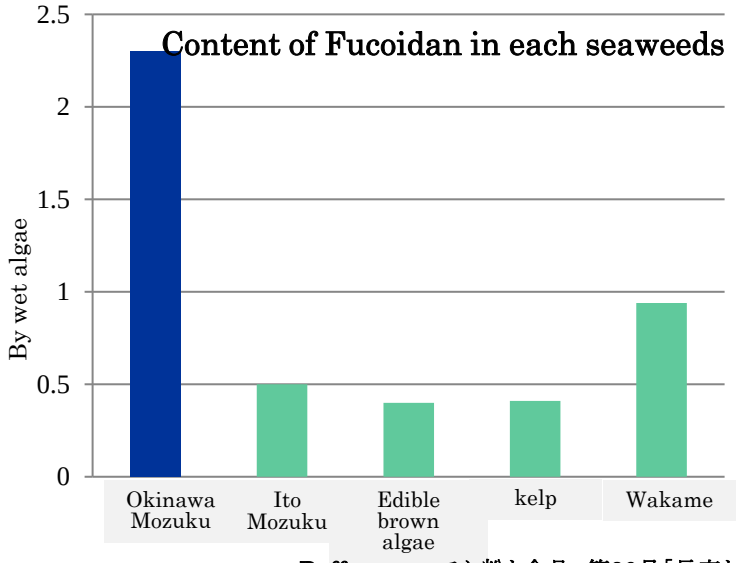
Fucoidan from Okinawa Mozuku

KANEHIDE BIO CO.,LTD.

What is Okinawa Mozuku?

Okinawa Mozuku *Cladosiphon okamuranus*

Mozuku is a brown alga which is classified under the family Chordariaceae and is an original species in only Okinawa islands. Eating mozuku habit has a long history however it was only for special areas and a period until established a farming mozuku skill even if in Okinawa. The farming skill had been developed after 1970's and now farmed 20 thousands tons of mozuku in Okinawa in a year, this amount means 90% in gross domestic production. Comparatively mozuku and other seaweeds, mozuku has a higher content of Fucoidan and further lower content of impurity as like alginic acid. This is to say Okinawa mozuku is excellent as a material of Fucoidan.



Refference：でん粉と食品　第29号「長寿と海藻」

Seaweed-eating culture in Okinawa

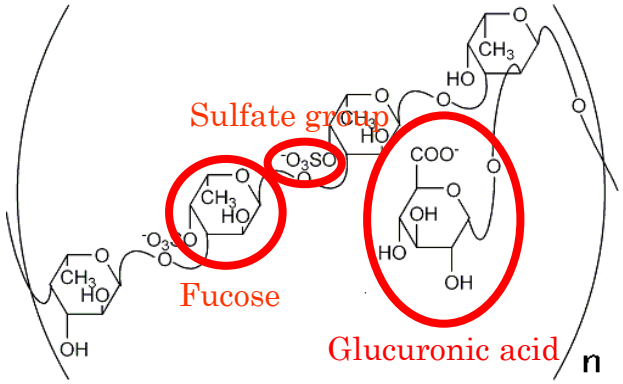
For Okinawan people which are surrounded by sea, seaweed is a important food source. Particularly in the spring tide we often gather seaweeds and eat them, we call this culture “hamakudari” in Japanese. Not only Okinawa, in Japanese meals, more than 20 species are used including a seaweed-based soup stock however these seaweeds are not eaten. In Okinawa we eat seaweeds with flied or pickled consequently we can take all of useful component of seaweeds. This may be a cause of the longevity island.

Okinawan people gathering seaweeds.

Okinawan traditional foods.

What is Fucoidan?

Fucoidan is a soluble viscosity polysaccharide which is contained in brown algae as like Mozuku, sea tangle and wakame. Compared with other algae, Mozuku has higher content and higher purity of Fucoidan.



Constitutional formula of Fucoidan extract from Mozuku

History of Fucoidan studies

1913	: Dr. Kylin from Uppsala University discovered Fucoidan into brown algae.
After 1950's	: Reported in blood anticoagulant activities of Fucoidan.
After 1960's	: Studied in component of Fucoidan from variety brown algae and chemical constitution.
After 1970's	: Reported in anti tumor activities.
1996	: Dr. Tako from Ryukyu University reported that Okinawa mozuku contain a lot of Fucoidan.

Bioactivity of Fucoidan based on studies

1. Anti tumor activity
2. Apoptosis induction activity against cancer cell
3. Immunostimulatory activity
4. Hypoglycemic activity
5. Blood pressure lowering effect
6. Anti-inflammation activity(for atopic dermatitis)
7. Antiviral activity (for WEE and HSV)

Kanehide Bio Fours points of Fucoidan

1. Using only Mozuku from Okinawa.
2. Fucoidan is high purity and produced with our original manufacturing method.
3. Nothing used any dextrin, 100% pure powder(we guarantee Fucoidan and sulfate group content)
4. Substantial evidence and guarantee of safety by human trials
5. Manufacturing products in a factory certified ISO22000 and GMP.
6. We have a lot of experience to export and domestic sell.

Manufacturing process

Continuous production in the Kanehide plant.

Receipt of material

Extraction by organic acid

Separation of solid and liquid

Ultrafiltration(salt and iodine)

Concentration

First inspection

Powderization

Second inspection(dosage form: powder, bulk)

Pockaging (dosage form: tablet, granule, cuspule, liquid)

Final Inspection

 Kanehide Bio Co., Ltd

Chemical analysis of Fucoidan from Mozuku

Item	Fucose content 1)	Uronic acid content 2)	Sulfate group content 3)	Molecular mass 4)	Fucoidan content 5)
Measured values[6)]	45.6 %	26.7 %	19.1 %	77,000	89.9 %
Value of standard	-	-	≧13 %	30,000～150,000	>85 %

1) Anthrone-sulfuric acid method　2) Carbazole sulfuric acid method
3) Ion chromatography　4) HPLC gel filtration technique
5) HPLC gel filtration technique　6) Average of 4 lots

Species of seaweeds	Characteristics of ingredient
Okinawa mozuku	Higher content of fucose compared with other seaweeds. It occupy 40% of sulfate group in fucose.
Kelp	More than 3 kinds of polysaccharide as Fucoidan. It contains more than 25% of sulfate group.
Wakame	It contains as amount as fucose and galactose which contains Sulfated fucogalactan.
Mekabu	It contains Sulfated polysaccharides which is a similarity with Wakame.

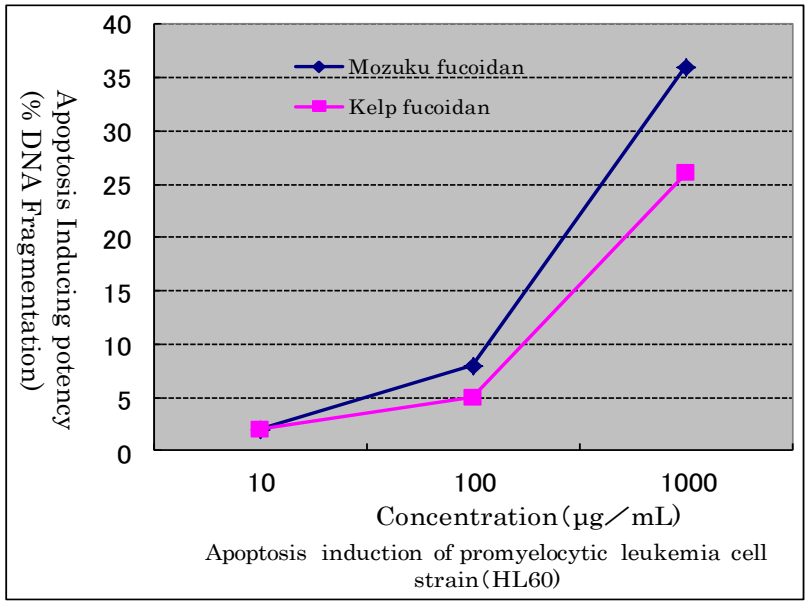
Bioactivation of Fucoidan from Okinawa Mozuku

1. Apoptosis inducing activity
2. Killing tumor cells activity
3. Anti-tumor activity (animal test)
4. Macrophage activation (immunostimulation)
5. Anti- atopic dermatitis activity(animal test)
6. Anti-obesity activity(animal test)
7. Immunostimulation activity (clinical examination)

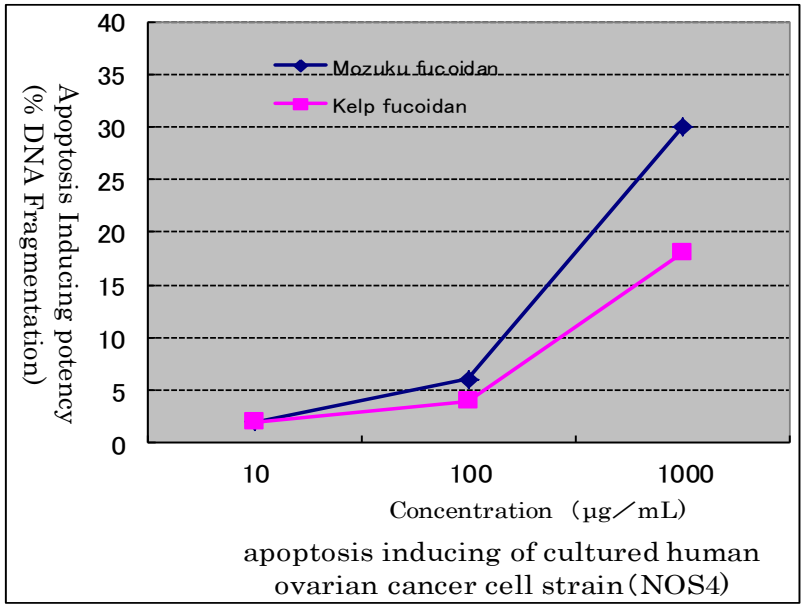
Apoptosis Inducing activity

【Test Method】

Added Fucoidan to HL60 （promyelocytic leukemia cell strain) and NOS4(cultured human ovarian cancer cell strain), evaluated the ratio of DNA fragmentation in cells as apoptosis inducing potency.



Apoptosis induction of promyelocytic leukemia cell strain（HL60）



apoptosis inducing of cultured human ovarian cancer cell strain（NOS4）

【Result】

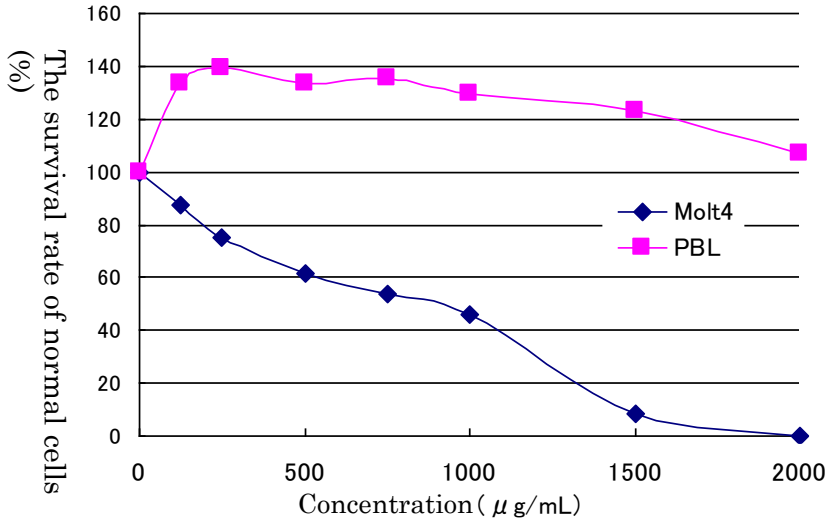
Measuring the apoptosis inducing activity with Fucoidan from Okinawa Mozuku and from kelp, it was found that Fucoidan from Okinawa Mozuku had a more significant apoptosis inducing effect than Fucoidan from kelp.

The data from result of joint research with Tokyo University of Science

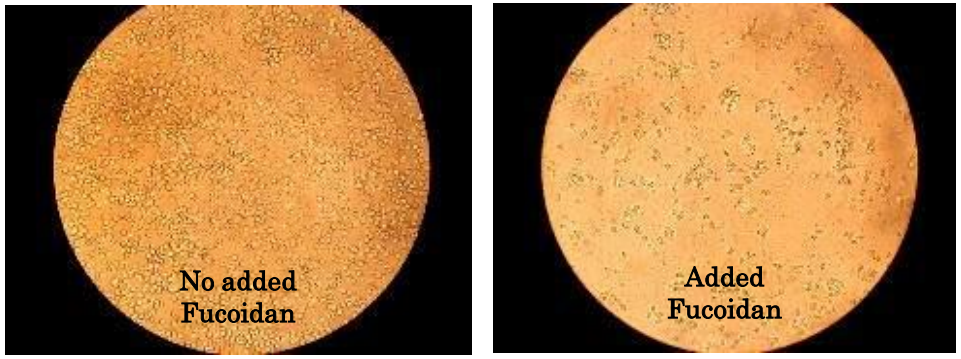
Killing tumor cells activity

【Test Method】

Using PBL (human lymphocyte) as normal cells and Molt-4(acute lymphoblastic leukemia) as continuous cancer cell from human, measuring a survival rate after adding Fucoidan.



GRAPH. The effect of Fucoidan with PBL and Molt-4



pic.) The comparison of multiplication of Molt-4 with no added fucoidan and added fucoidan

【Result】

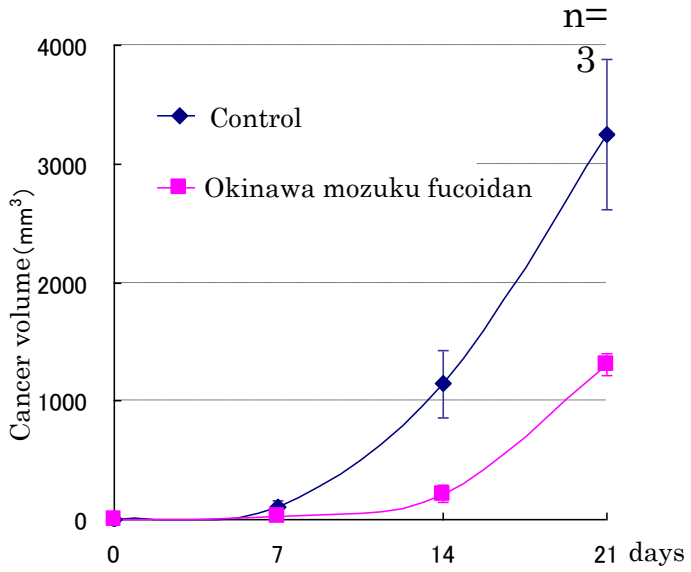
It is not found a viability loss of normal cells (PBL) whereas the survival rate of cancer cells (Molt-4) was controlled depending on concentration. As a result we suppose Okinawa mozuku fucoidan to have a specific effect against cancer cells.

The data by Kanehide Bio Co., Ltd.

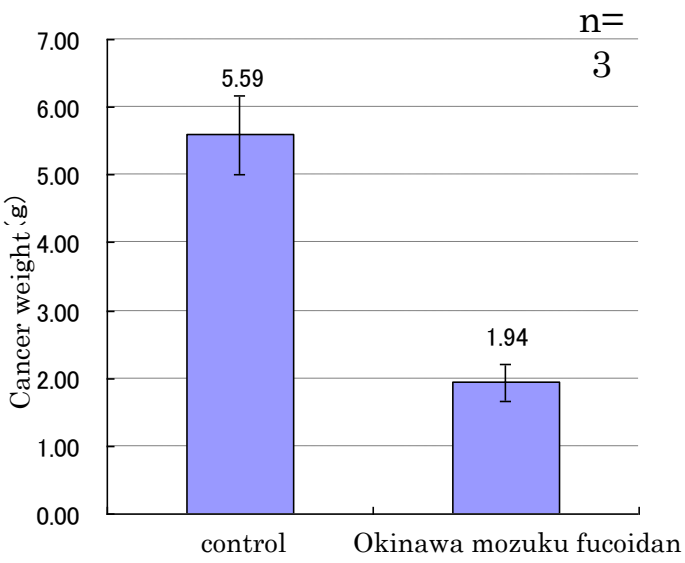
Anti-tumor activity (animal test)

【Test Method】

Tested the tumor cells (mouse sarcoma cell strain :Sarcoma180) growth inhibition with nude mouse. after administering cancer cells in subcutaneous. Oral inoculating 100 mg per kg of Okinawa mozuku fucoidan for 21 days once per day after administering cancer cells in subcutaneous.



Effect against cancer growth



Effect against cancer weight（21days）

【Result】

It is verified a significant difference （p＜0.005） of Okinawa mozuku fucoidan compared with the control on the 14th day and on the 21st day verified a significant difference （ p ＜0.0005） .as a result of the test, it is verified that Okinawa mozuku fucoidan controls a growth of cancer cells.

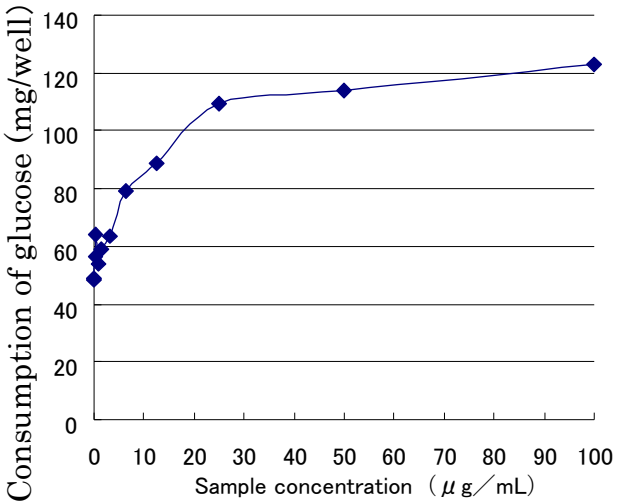
The data from result of joint research with Prefectural University of Kumamoto

Macrophage activating potency (immunostimulation)

【Test Method】

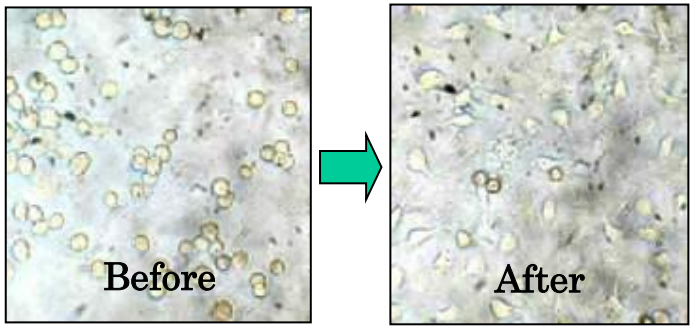
Macrophage is a cell which has a excellent phagocytic activity, it plays an important part likewise not only digesting foreign matters in a body, but also releasing cytokines or active oxygen, acting to bridge and accommodating to immunoreaction such as antigen presentation, attacking or removing cancer cells and so on.

In addition it is known as glucose metabolism system participate when macrophages produce active oxygen. Consequently we can estimate that the higher consumption of glucose, the higher production quantity which means the higher immunostimulating activity.



Data: Activation of macrophage indicated by consumption of glucose

Added 100μg/mL of fucoidan



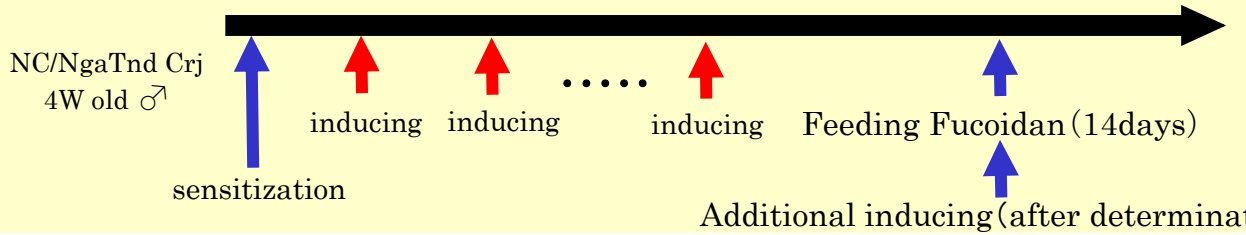
It is observed spreading dilatation which we can see when macrophage activates.

【Result】

Measuring activation of macrophage indicated by consumption of glucose, we confirmed that Okinawa mozuku fucoidan has a enhancing glucose consumptive activity which means Okinawa mozuku fucoidan has immunostimulating activity.

Anti atopic dermatitis activity (animal test)

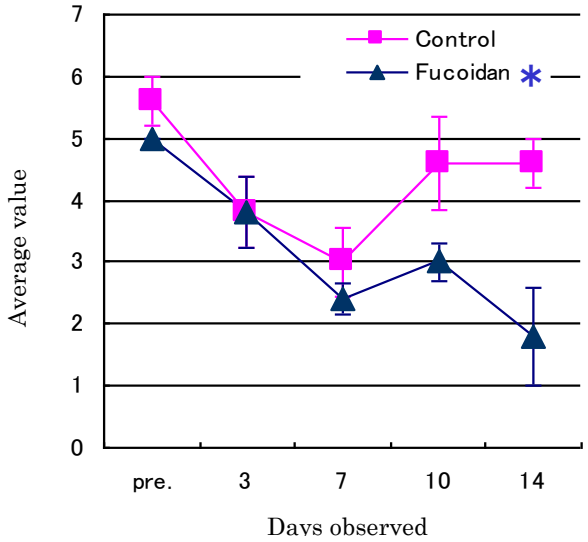
【Method】



Inspection Item
1. Pruritus cutaneous
2. Redness and bleeding
3. Edemas
4. Excoriation and tissue deficit
5. Incrustation and dryness

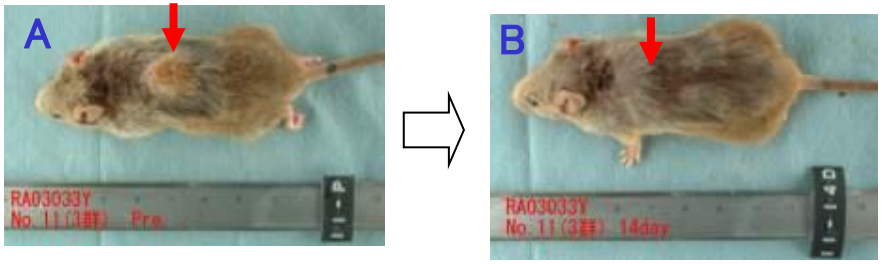
Determination

Categorizing 4 steps from asymptomatic to high symptom and determining chronologically the average value of total rating as seriousness.



Pic) influences regard to atopic dermatitis.

＊: Compared with control, there was $p < 0.05$ of significant difference.



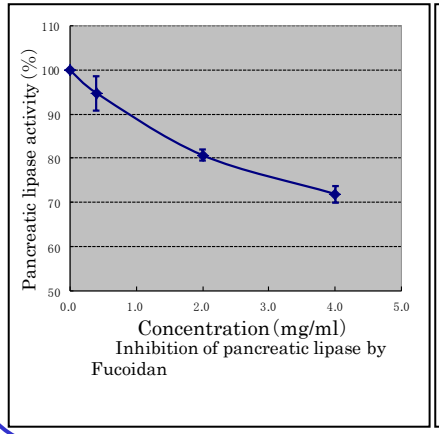
Pic) influences regard to atopic dermatitis

A: Starting day　　B: Day 14

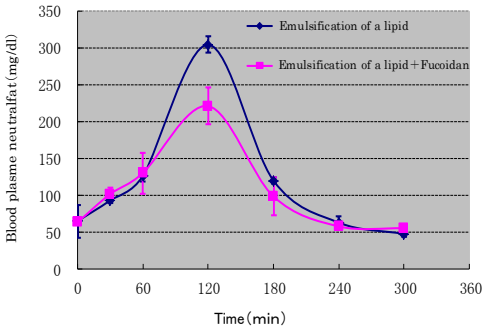
【Result】

Determined improvement for dermatitis with oral administration of fucoidan in atopic dermatitis mice.

Anti-obesity activity (animal test)



Inhibition of pancreatic lipase by Fucoidan



Suppression of neutral fat increases by Fucoidan n=4

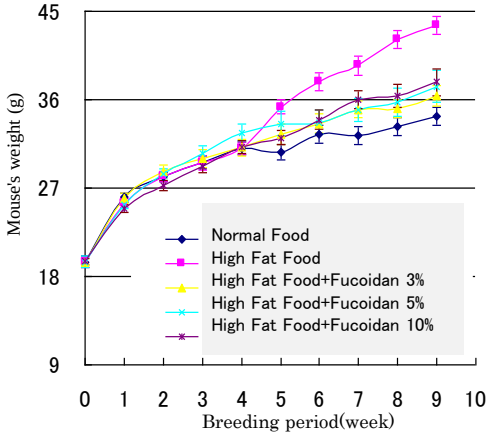
Inhibition of pancreatic lipase activity and Activity of reducing neutral fat

Fucoidan extracted from Okinawa Mozuku inhibited the activity of pancreatic lipase which is a lypolytic enzyme. In experiments of oral administration in rats, inhibited for rise in blood neutral fat after administration of lipid.

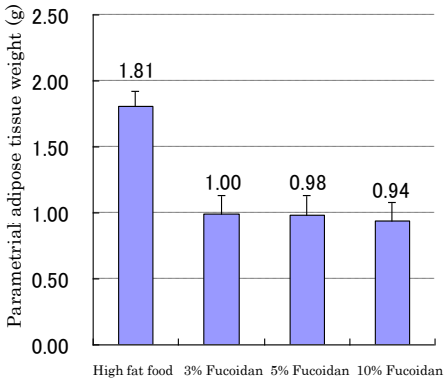
Preventing weight gain

【Result】

Maintained the weight of mouse and Parametrial Adipose Tissue by adding Fucoidan to high fat food.



(pic.)Effect of Fucoidan on body weight in mouse with high fat food



(pic.)Effect of Fucoidan on parametrial adipose tissue weight in mouse with high fat food

Immunostimulation activity (human test : mucous immune in oral)

Secretory IgA（s-IgA）

- ✓ Main immunoglobulin found in mucous secretions and it removes foreign matters.
- ✓ The first line of defense against bacteria, food residues, yeast, parasites and viruses and it prevents invasion of them.
- ✓ In the oral, s-IgA in saliva defends against bacteria and viruses infect to upper airway.

Measuring amount and speed of s-IgA secretions as an index of oral immune

- ✓ Sampling saliva is simple, easy noninvasive.
- ✓ The measuring is a useful as a tool for observing condition of healthy individual’s immune system.

Immunostimulation activity (human test : mucous immune in oral)

Research subjects

- ✓ 22 healthy people（11 male, 11 female）
- ✓ From 20 years old to 65 years old（average 42.3±10.4 years old）
- ✓ Slowing secretions of secretory IgA(s-IgA)

Test sample

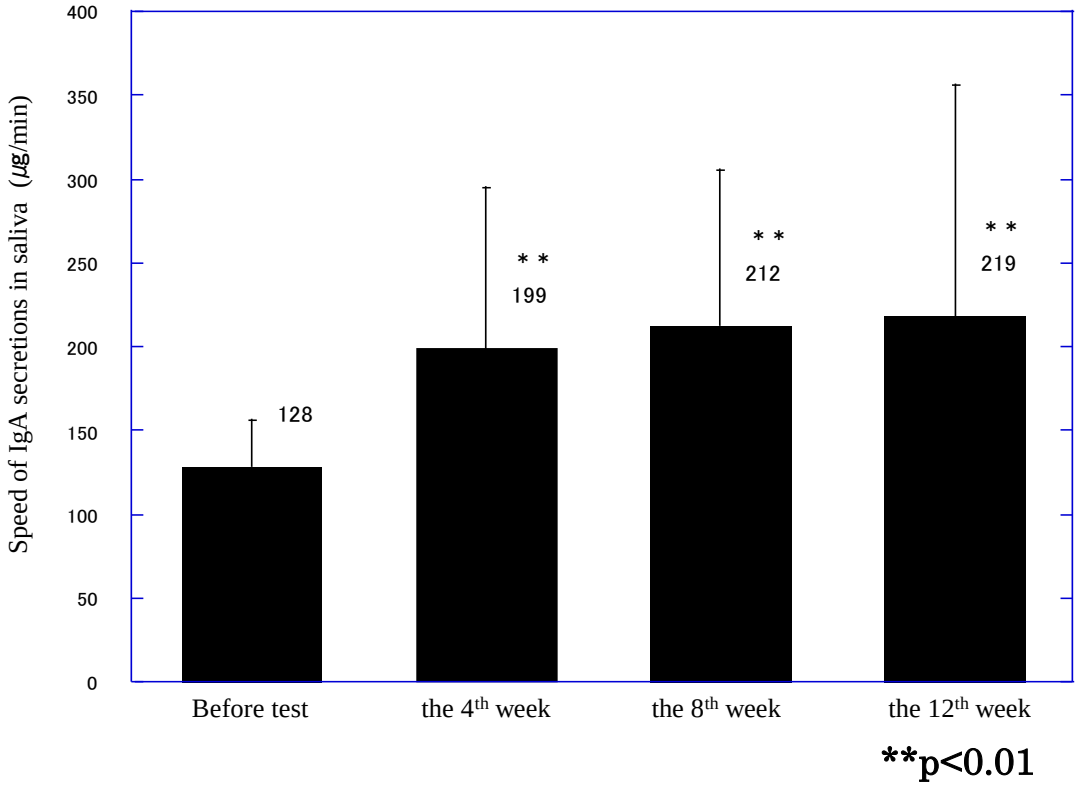
- ✓ Fucoidan in capsule
- ✓ 5 capsules /day

（contained 1,175mg of Okinawa mozuku extract）

How to test

- ✓ Taking 5 capsules every day for 12 weeks.
- ✓ Measuring speed of s-IgA secretions in saliva at the 4th week, the 8th week and the 12th week.
- ✓ Urinalysis and blood inspection on the 12th week.

Immunostimulation activity (human test : mucous immune in oral)



- ✓ Comparing with before test, the speed of s-IgA secretions in saliva at the 4th week, the 8th week and the 12th week significantly increased.
- ✓ Expected immune boosting effect by taking fucoidan from Okinawa mozuku.

Safety (Data of clinical examination in before and after taking)

＜Hematology test＞

Item	Unit of measure	Standard	Before	12th week
WBC	(/μL)	3300 - 9000	6013.6 ± 2065.9	5904.5 ± 1839.9
RBC	($\times 10^4$/μL)	Male：430 - 570 Female：380 – 500	471.3 ± 49.1	464.4 ± 45.9
Hb	(g/dL)	Male：13.5 – 17.5 Female：11.5 – 15.0	14.06 ± 1.69	13.87 ± 1.85
Ht	(%)	Male：39.7 – 52.4 Female：34.8 – 45.0	43.49 ± 4.42	42.44 ± 4.60**

＜Biochemical test＞

Item	Unit of measure	Standard	Before	12th week
AST	(U/L)	10 - 40	19.9 ± 4.6	18.7 ± 5.0
ALT	(U/L)	5 - 45	18.0 ± 7.2	16.3 ± 8.5
LDH	(U/L)	120 - 240	166.9 ± 29.7	158.0 ± 28.5**
γGT	(U/L)	Male：80以下 Female：30以下	25.0 ± 13.3	22.4 ± 10.8*
ALP	(U/L)	100 - 325	213.4 ± 75.0	209.4 ± 73.0
TB	(mg/dL)	0.2 - 1.2	0.73 ± 0.23	0.78 ± 0.29
TP	(g/dL)	6.7 - 8.3	7.39 ± 0.47	7.28 ± 0.46
Alb	(g/dL)	3.8 - 5.2	4.59 ± 0.29	4.49 ± 0.31
Cr	(mg/dL)	Male：0.61 - 1.04 Female：0.47 - 0.79	0.758 ± 0.190	0.807 ± 0.180**
UN	(mg/dL)	8.0 - 20.0	12.35 ± 2.90	12.52 ± 3.07
UA	(mg/dL)	Male：3.8 - 7.0 Female：2.5 - 7.0	5.07 ± 1.18	5.21 ± 1.47
GLU	(mg/dL)	70 – 109	83.0 ± 5.6	84.0 ± 8.8
TG	(mg/dL)	30 – 149	83.3 ± 30.3	100.8 ± 76.7
TC	(mg/dL)	120 – 219	202.7 ± 40.8	188.0 ± 32.9**
HDL-C	(mg/dL)	Male：40 - 85 Female：40 – 95	65.4 ± 16.0	62.0 ± 15.9*
LDL-C	(mg/dL)	65 - 139	119.0 ± 32.2	107.0 ± 30.5*

the mean value ± standard deviation,**p<0.01, *p<0.05 vs before

It is founded some data fluctuation and adverse event (as like cavities) however the symptoms are mild and be detected that the symptoms don't have relevance to Fucoidan.

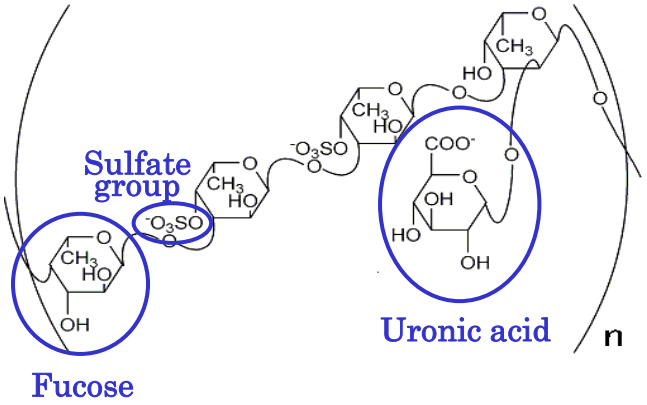
Safety of Fucoidan from Okinawa Mozuku

22 healthy people　approx. 1.2g/day×12 weeks・・・・NOTHING PROBLEM

Absorption of Fucoidan

Activity of Fucoidan

- **Immunostimulation activity**
- **Anti tumor activity**
- **Anti virus activity**
- **Anti allergy activity**
- **Anti blood coagulating activity**
- **hypocholesterolemic activity and etc.,**

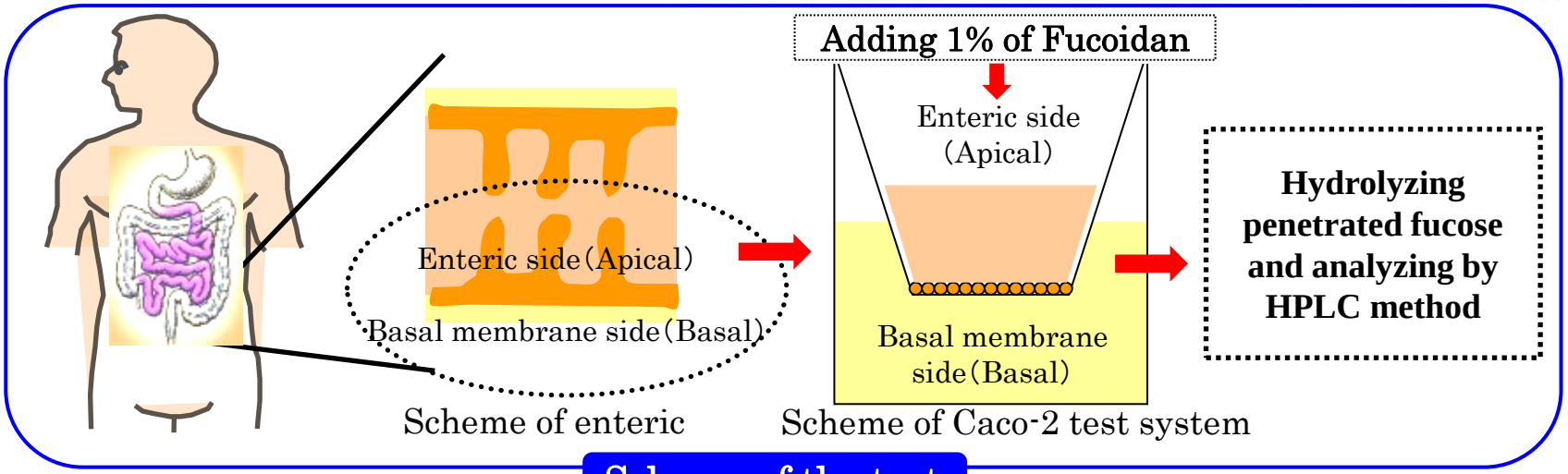


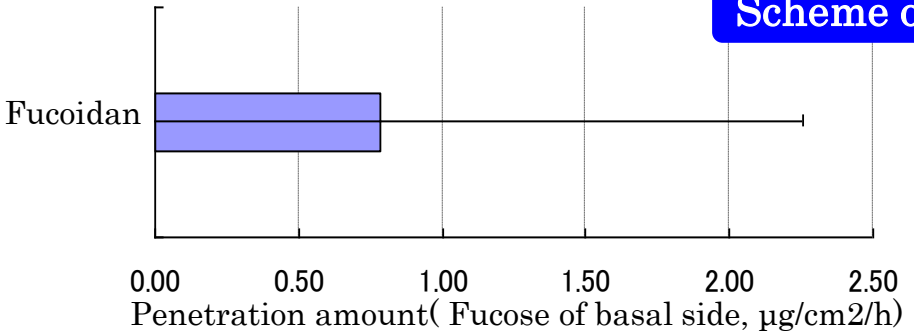
(pic.) Constitutional formula of Fucoidan from Okinawa mozuku

Since Most of these reports are tests in vitro, absorbable is precondition for these activity.

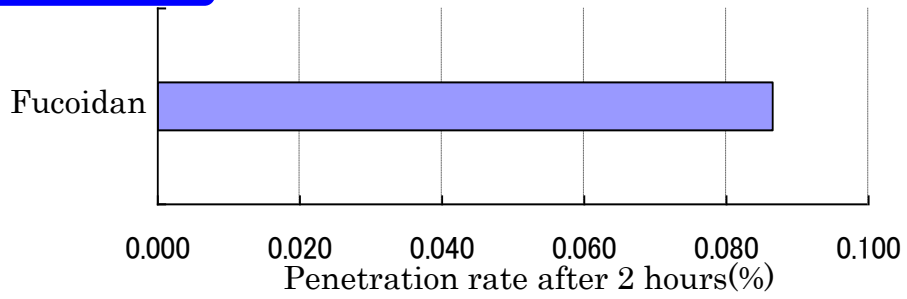
As Fucoidan is a macromolecule substance, generally considered most of Fucoidan might not absorb intestinal.

Predicting absorbability in model test with the small intestine membrane





Pic.) Penetration amount for Fucoidan



Pic.) Penetration rate for Fucoidan

【Result】

By Prospective study for absorbability of Fucoidan with intestine membrane model, we founded Fucoidan through the small intestine membrane. It means we suggested Fucoidan is absorbable in intestinal .

Safety <Animal test and clinical examination (Excess intake)>

4 week repeat dose studies using rats

Test facility :	CRO Rabiton Co., Ltd.
Subjects :	Wister rats, 10 each group (5 male, 5 female)
Test Sample :	Fucoidan Extract Powder Capsule 1. 135mg/kg（low dosage） 2. 270mg/kg（high dosage）
Test method :	Oral administration once in a day for 4 weeks

- ✓ **No abnormalities found in biochemical examination of the blood or hematological assessment.**
- ✓ **No difference between groups administered low dosage and high dosage.**

Prolonged consumption tests of fucoidan by healthy individuals

Test facility :	Tokyo Medical and Dental Univ.
Subjects :	Staff of Kanehide Bio Co., Ltd. and Tokyo Medical and Dental University, 35 healthy people (19 male, 16 female)
Test Sample :	Fucoidan Extract 50mL/bag (6g of Fucoidan) 1 bag per day for 1 to 3 month
Test method :	

- ✓ **There was no significant difference in body weight, blood tests, or urinalysis tests before and after taking Fucoidan. No adverse experiences were recorded.**
- ✓ **Immune function tended to be elevated.**

Confirmed evidence (conference presentation)

Year	Title of the presentation and name of the event
2003	・Japanese Cancer Association 62nd General Meeting "Potential T cell Leukemia Treatments with Fucoidan from *Cladosiphon okamuranus* "
	・American Society of Hematology 45th General Meeting "Apoptosis Induced by Fucoidan from *Cladosiphon Okamuranus* Tokida in HTLV-I-Infected T-Cell Lines and Primary ATL Cells."
2004	・2nd Young Researchers Forum on Infectious Diseases "Potential T cell Leukemia Treatments with Fucoidan from *Cladosiphon okamuranus* "
	・Japanese Cancer Association 63rd General Meeting "Anticancer effect of Fucoidan from Okinawan *Cladosiphon okamuranus* on Gastric Cancer Cell Lines" "Inverstigation of the Anticancer Effect due to differences in Molecular Weight and Elements of Fucoidan from Okinawan *Cladosiphon okamuranus* "
2005	・Japanese Cancer Association 64th General Meeting "Anticancer effect of Fucoidan from Okinawan *Cladosiphon okamuranus* on Sarcoma Cell Lines in Mice"
	・Japan Society Nutrition Food Science 59 "A factor of Fucoidan from Okinawa mozuku relating to immunology"
2006	・Japanese Cancer Association 65nd General Meeting "Fucoidan extracted from Okinawan *Cladosiphon okamuranus* and the Mechanism for Inducing Mouse Macrophage NO Production" "Anticancer effects of Okinawan *Cladosiphon okamuranus*"

Confirmed evidence (scientific reports and theses)

Year	Title of reports or theses
2004	・Vol. 66, No.1・2, The Japanese Journal of Constitutional Medicine “Anti-obesity Effects of Fucoidan Prepared from *Cladosiphon Okamurananus tokida* (Okinawamozuku)
2005	・Nutrition and cancer, 52(2), 189-201 「Fucoidan extracted from *Cladosiphon okamuranus* Tokida induces apoptosis of human T-cell leukemia virus type1-infected T-cell lines and primary adult T-cell leukemia cells」
2005	・Japan Society Nutrition Food Science 58: 273-280(2005) “Biological Properties of Fucoidans Differing in Molecular Weight and Composition: Effects on Lymphocyte Proliferation and Immunological Function”
2011	・Antiviral Therapy 2011;16:89-98 「Fucoidan therapy decreases the proviral load in patients with human T-lymphotropic virus type-1-associated neurological disease」
2012	・World J Gastroenterol 2012 May 14;18(18):2225-2230 「Beneficial effects of fucoidan in patients with chronic hepatitis C virus infection」
2012	・INTERNATIONAL JOURNAL OF ONCOLOGY 40: 251-260, 2012 「Anti-tumor activity of fucoidan is mediated by nitric oxide released from macrophages」
2013	・Marine drugs, 11, 4267-4278; doi: 10.3390/md11114267 「Cytotoxic Effects of Fucoidan Nanoparticles Against Osteosarcoma」
2018	・INTERNATIONAL JOURNAL OF ONCOLOGY 53: 801-814, 2018 「Mitotic kinase PBK/TOPK as a therapeutic target for adult T-cell leukemia/lymphoma」

