

“CELABIO[®]-F”: a fermented plant origin material having comparable effect to placenta

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SUMMARY

At the contemporary aging society, the need for health-oriented and safe anti-aging products is getting stronger. One of the typical materials to meet that condition is placenta, which is widely used for many products.

Taking the social trend to request safer plant origin materials having comparable effect to placenta into consideration, we have been looking for new materials and have found that the mixture of rice bran extract and soybean extract fermented by *Bacillus subtilis var. natto* possesses multiple functions similar to placenta. So we have developed it as a functional food material (Trade name: CELABIO[®]-F).

Through the ingestion test of CELABIO[®]-F, it was confirmed that the material has effect on beauty, ameliorating climacteric complaints, improving blood stream, reducing oxidative stress in the body, anti-fatigue, hepatic function improvement, blood triglyceride level lowering and so on.

Introduction

The placenta is an organ of the mammal that connects the developing fetus to the uterine wall to allow nutrient uptake, thermo-regulation, waste elimination, and gas exchange via the mother's blood supply during pregnancy. It is rich in amino acids, vitamins, minerals, etc. as well as proteins, lipids and saccharides.

Mainly its extract is widely used for pharmaceuticals, foods such as nutritional supplements & beverages and cosmetics.

In recent years, the sales of placenta extract containing products are growing. Especially because of the existence of cell proliferation factor, its application to the nutritional supplement and cosmetics as the anti-aging material is increasing.

Its multiple functions other than beauty effect have been recognized and the research

results on climacteric symptom improvement, hepatic function improvement, etc. have been reported¹⁾⁻¹²⁾.

In the past, placenta extract from cows was widely utilized for food and cosmetics, however owing to BSE (bovine spongiform encephalopathy) problem, the placenta sources have almost been changed to pigs or horses.

Owing to the recent tendency toward safety, placenta –like ingredients from marine organisms such as salmon ovarian membrane and/ or from flowering plant placenta such as melon and rose are on the market¹³⁾.

In case of pharmaceuticals, placenta derived only from human is used.

Taking the social situation mentioned above into consideration, we have developed a new placenta-like material for food use, that is, the mixture of rice bran extract and soybean extract fermented by *Bacillus subtilis var. natto* (Trade name: CELABIO[®]-F).

Here, we introduce the confirmed effects of CELABIO[®]-F in case of oral ingestion on beauty, ameliorating climacteric complaints, improving blood stream, reducing oxidative stress, anti-fatigue, hepatic function improvement and so on.

1. The mixture of rice bran extract and soybean extract fermented by *Bacillus subtilis var. natto*, “CELABIO[®]-F”

“CELABIO[®]-F” is the powder obtained by the filtration of the mixture of rice bran extract and soya bean extract fermented by *Bacillus subtilis var. natto*, and then the addition of dextrin.

The powder contains the fermented material by 20% on solid ratio.

2. Beauty effect

2.1. Fibroblast activating effect

Normal human fibroblast was inoculated on microplate and incubated for 24 hours. Then the medium was replaced to the medium containing “CELABIO[®]-F” and further incubated for 48 hours. Later cell numbers were counted by MTT assay.

Then it was confirmed that “CELABIO[®]-F” possesses cell activating effect which is superior to that of porcine placenta extract (solid content: 33%) at the lower concentration (Fig.1).

2.2. The activity to accelerate production of Human Type I collagen

Normal human fibroblast was inoculated on microplate and incubated for 24 hours. Then the medium was replaced to the medium containing “CELABIO[®]-F” and further incubated for 72 hours.

Later collagen quantity in the supernatant of the incubated medium was measured by Human collagen, Type I, ELISA Kit.

It was confirmed that “CELABIO®-F” possesses statistically significant higher collagen production activity than control (Fig.2).

2.3. Production promoting activity for hyaluronic acid

Normal human fibroblast was inoculated on microplate and incubated for 24 hours.

Then the medium was replaced to the medium containing “CELABIO®-F” and further incubated for 72 hours. Later hyaluronic acid quantity in the supernatant of the incubated medium was measured by Hyaluronan DuoSet, ELISA.

It was confirmed that “CELABIO®-F” possesses statistically significant higher hyaluronic acid production promotion activity than control (Fig.3).

2.4. Skin-beautifying effect by oral ingestion (1)

Subject was healthy 13 adults of both sexes (20-50 years old).

Subjects ingested 500mg of “CELABIO®-F” every day for 28 days and the change of the moisture content and the elasticity of skin at the cheek of the face before and after the ingestion was checked.

Skin diagnosis was implemented using CYBER SKIN CHECKER PT of JAPAN GALS Co. Ltd.

The moisture content and the elasticity of the skin was found to be improved by continuous ingestion of “CELABIO®-F” and the result of questionnaire shows that 77% of the subjects felt skin condition improvement (Fig.4,5).

The examples of skin condition improvement by microscopic observation are shown in Fig.6.

Before the ingestion, there were skin parts where neither cristae cutis nor sulci cutis could be seen, but after ingestion both could be seen clearly, which is the proof of texture improvement.

2.5. Skin-beautifying effect by oral ingestion (2)

Subject was healthy 8 adults of both sexes (30-50 years old).

Subjects ingested 495mg of “CELABIO®-F” every day for 28 days and the change of face skin condition before and after the ingestion was checked.

Using skin image analysis equipment, VISIA® of Integral Corporation, skin condition was measured and the result was compared with the total score of both right and left sides of the face.

Continuous ingestion of “CELABIO®-F” resulted in the significant improvement

and/ or the tendency of improvement in brownish spot (by melamine), pore, texture and spot (by ultraviolet ray) (Fig.7).

3. **The effect on ameliorating climacteric complaints**

13 Japanese ladies who realize climacteric complaints and whose Kuppermanmenopausalindex score in medical interview is relatively high were screened as subjects for the test.

Subjects ingested 495mg of “CELABIO®-F” every day for 8 weeks and the change of Kuppermanmenopausalindex before and 4 & 8 weeks after the ingestion was compared.

After the injection of “CELABIO®-F” for 4 or 8 weeks, the total score of Kuppermanmenopausalindex got lowered with statistical significance in the degree of severity from “severe” (35 or more) before ingestion to “moderate” (21-34) and to “mild” (16-20), by which the improvement of climacteric complaints was confirmed (Fig.8).

Statistically significant improvements with time were recognized in all the symptom - complexes (vasomotor nerve disturbance-like symptom, sensory disturbance-like symptom, asomnia, nervousness, depression, dizziness, general malaise, arthralgia/ muscle soreness, headache, rapid pulse, formication) (Table1).

4. **The effect on blood stream improvement**

Subject was healthy 8 adults of both sexes (20-50 years old).

Subjects ingested 495mg of “CELABIO®-F” every day for one week and the change of skin condition before and after the ingestion was surveyed.

The function of blood peripheral circulation was checked with Blood Circulation Checker and waveform pattern was converted to the score for evaluation.

At the same time, blood pressure was measured.

The improvement tendency of blood peripheral circulation function was recognized one hour after one time ingestion and further the statistically significant improvement was recognized after the one week consecutive ingestion (Fig.9),

Concerning blood pressure, systolic blood pressure showed the tendency to be lowered one hour after one time ingestion and it was lowered to the statistically significant proper range level after one week consecutive ingestion (Fig.10).

5. **Anti-fatigue effect**

Subject was healthy 10 university male students (all were athletes).

One hour before the test, subjects ingested 10g of “CELABIO®-F” mixed with soft drink, and bicycle ergometer test was made at an exercise tolerance of (body weight x 7.5%).

Exercise tolerance test was made as follows: (10 seconds pedalling at maximum speed followed by 50 seconds rest) x 30 times’ repetition.

Later, as the control, exercise tolerance test was made in the same way after ingesting only soft drink without “CELABIO®-F” addition.

Lactate concentration in the blood was measured before and after exercise tolerance test.

By the ingestion of “CELABIO®-F”, it was observed that power lowering was suppressed from 10minutes after exercise tolerance start and then exercise performance was improved compared with the control (Fig.11).

The tendency that lactate concentration in the blood gets lower in case of the ingestion of “CELABIO®-F” compared with the control was observed (Fig.12).

6. **The in vivo effects on anti-oxidative stress and triglyceride lowering**

Subject was healthy 18 adults of both sexes (20-60 years old).

Subjects ingested 479mg of “CELABIO®-F” every day for 12 weeks and the change of both titled effects before and after the ingestion was surveyed.

As the oxidative stress biomarkers, 8-OHdG (8-hydroxydeoxyguanosine) and carbonylated protein in the blood were determined.

By the consecutive ingestion of “CELABIO®-F”, the concentration of both 8-OHdG and carbonylated protein in the blood were decreased with statistical significance 4 weeks after ingestion, which showed the in vivo oxidative stress suppression (Fig.13-14).

And also, through this oral ingestion test, the lowering effect of triglyceride in the blood was recognized. The decrease was recognized in 8th week after ingestion and the statistically significant decrease was confirmed in 12th week after ingestion (Fig.15).

7. **Liver function improvement effect (acetaldehyde metabolism acceleration effect)**

7.1. In vitro test using human liver cell line

Chang liver cell was used as human liver cell line.

To the cultured solution of Chang Liver cells, either 1) mixture of rice bran extract and soybean extract,2) mixture of rice bran extract and soybean extract

fermented by *Bacillus subtilis var.natto*, 3) curcumin (10mg/ml), 4) alanine (10mg/ml), 5) ornithine (10mg/ml) or 6) glutathione (5mg/ml) was added with twofold serial dilution. After the addition, the solutions were allowed to stand for one hour and then the activities of aldehyde dehydrogenase were determined.

In case of 2) mixture of rice bran extract and soybean extract fermented by *Bacillus subtilis var. natto*, very high promotion effect of aldehyde dehydrogenase activity was recognized. The fermentation heightened the activity, which was found stronger than that of curcumin, the effective ingredient of turmeric (Fig. 16).

7.2. Human liver function improvement effect by oral ingestion (1)

Two healthy male subject ingested water, beverage containing turmeric (equivalent to 30mg of curcumin) and 100ml of 5% "CELABIO®-F" aqueous solution.

30 minute after ingestion, 700ml of beer (alcohol content: 5%) was taken over 30 minutes and acetaldehyde concentration in saliva was determined after 1, 3 and 5 hours.

Acetaldehyde concentration change with time showed that the metabolism of acetaldehyde generated after alcohol ingestion is accelerated by "CELABIO®-F" (Fig.17).

7.3. Human liver function improvement effect by oral ingestion (2)

Subject was healthy 13 adult male.

Test method was exactly the same as that of 7-2 "Human liver function improvement effect by oral ingestion (1)" and the effect was assessed by acetaldehyde concentration in saliva.

Grouping was made as shown in Table 2 and each test substance was ingested with 100ml of water.

This test was made by the cross-over study with each subject's participation in several test groups.

It was recognized that "CELABIO®-F" ingested alone or simultaneously with curcumin contributes to the acceleration of acetaldehyde metabolism, so that liver function improvement can be stronger than that of other materials which can be expected to have the same function (Fig.18).

Those data so far obtained show the possibility to prevent "hangover" by "CELABIO®-F" ingestion.

8. Conclusion

Placenta extract is reported to be effective as the ingredient of cosmetics on the improvement of texture, pore and red flare and also is suggested to have the potential of the dermal inflammation remedy, melanin formation suppression and skin cell turnover promotion.

And also when used as the ingredient of health foods, placenta is shown to have the potential of climacteric symptom improvement, liver function activation, anti-fatigue and nutritional fortification.

At the current aging society, people are getting more and more health conscious. Especially, products emphasizing the advantage of “anti-aging effects” are strongly demanded.

From this kind of circumstance, placenta extract is widely used as anti-aging ingredient for both cosmetics and health foods because of its multiple functions and its market shows steady growth.

Formerly, extract from the bovine placenta was used for health foods and cosmetics, however, since Bovine Spongiform Encephalopathy (BSE) was found to be a fatal neurodegenerative disease in cattle that may be passed to humans who have eaten infected flesh, extract of domestic animal placenta other than cow has been used. Goat is a kind of ruminant animals and so its placenta extract usage is also worried about the neurodegenerative disease in human like the case of BSE.

Therefore, placenta extracts of pigs and horses are currently used, however the need for safer plat-derived materials which possess functions similar to animal placenta extract is increasing.

“CELABIO®-F” we introduced here is the mixture of rice bran extract and soybean extract fermented by *Bacillus subtilis* var. natto and possesses various functions similar to animal placenta such as beauty effect (under patent application), ameliorating climacteric complaints (under patent application), improving blood stream, reducing oxidative stress in the body, anti-fatigue action (Patent No. 5855357), improving hepatic function and so on. It can be expected as the safe plant origin/ placenta-like material which meets the social needs to use safe material.

Since the various functional assessment of “CELABIO®-F” introduced here has been done on pilot scale level and the effective ingredients & the mechanism of the function are not known, we plan further detailed study.

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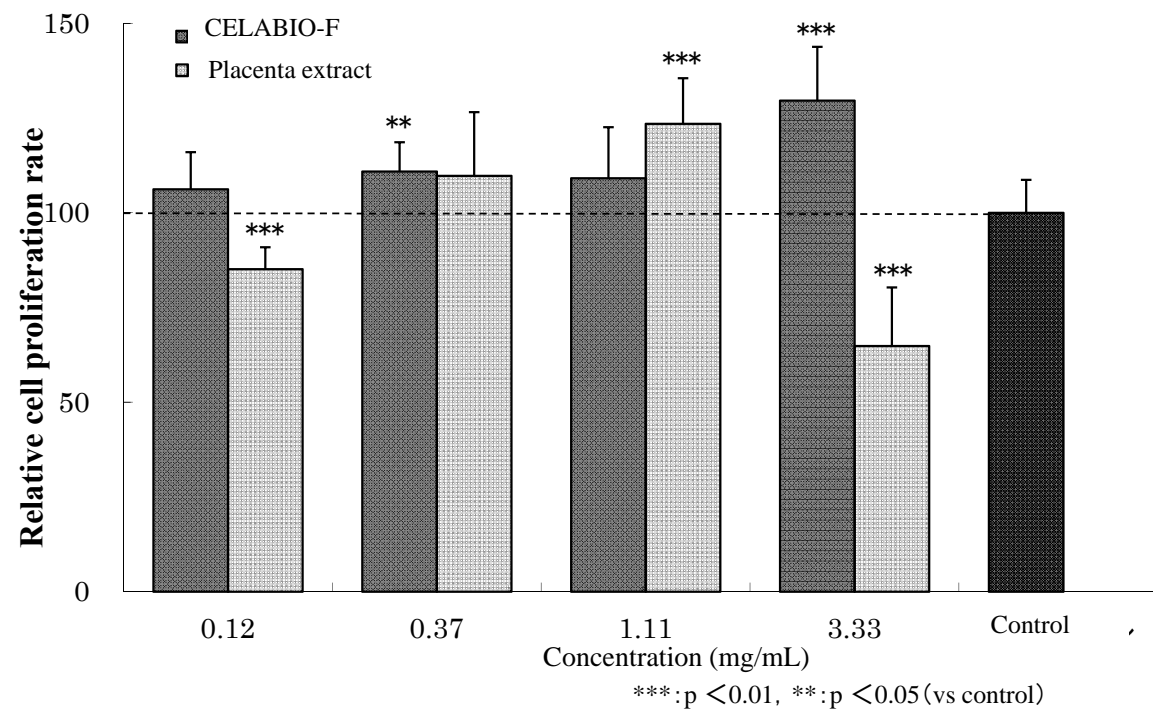


Fig. 1 Activator action on fibroblast

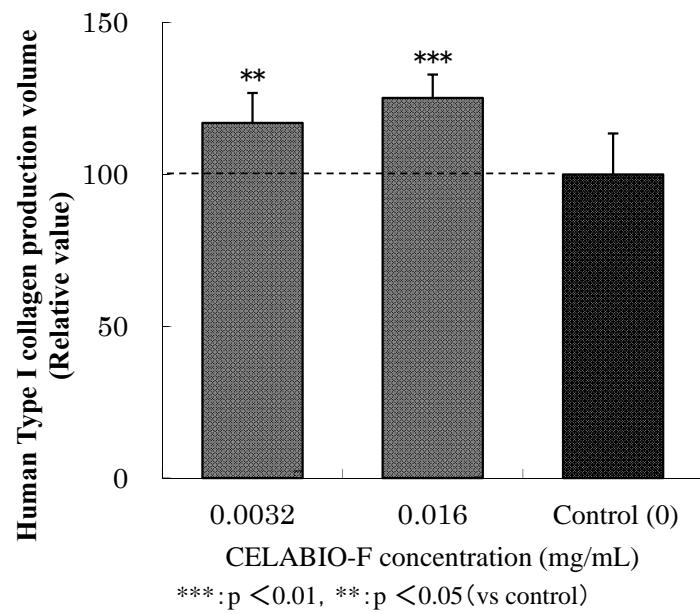


Fig. 2 The activity to accelerate production of Human Type I collagen

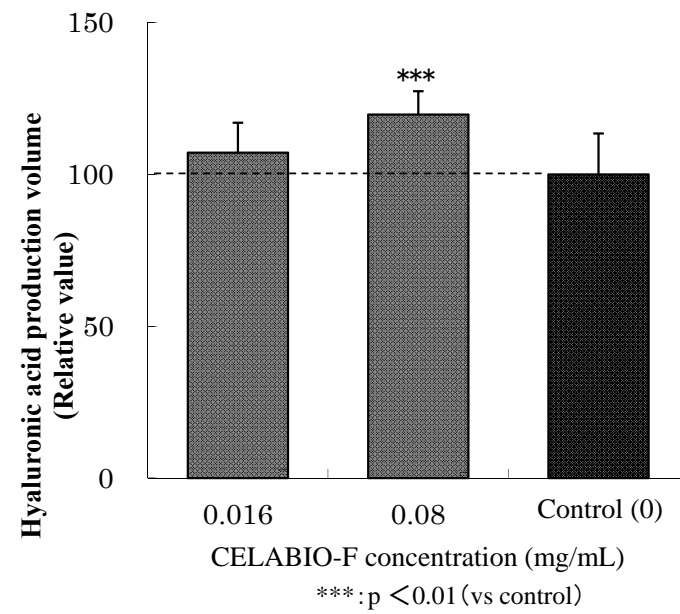


Fig. 3 The activity to accelerate production of Hyaluronic acid

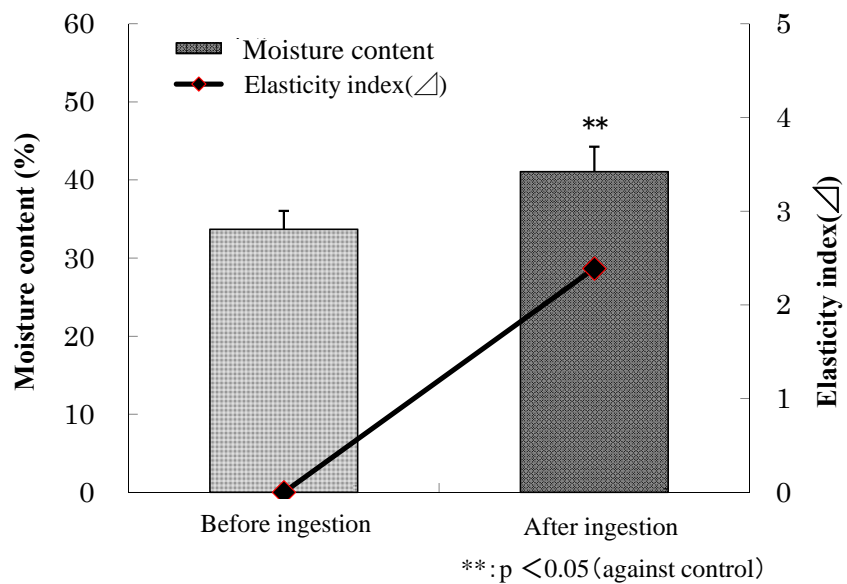


Fig.4 The change of moisture content and elasticity of skin (cheek)

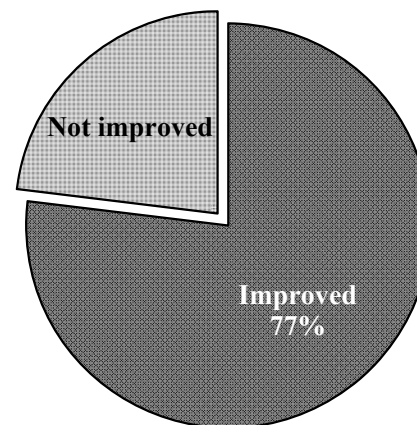
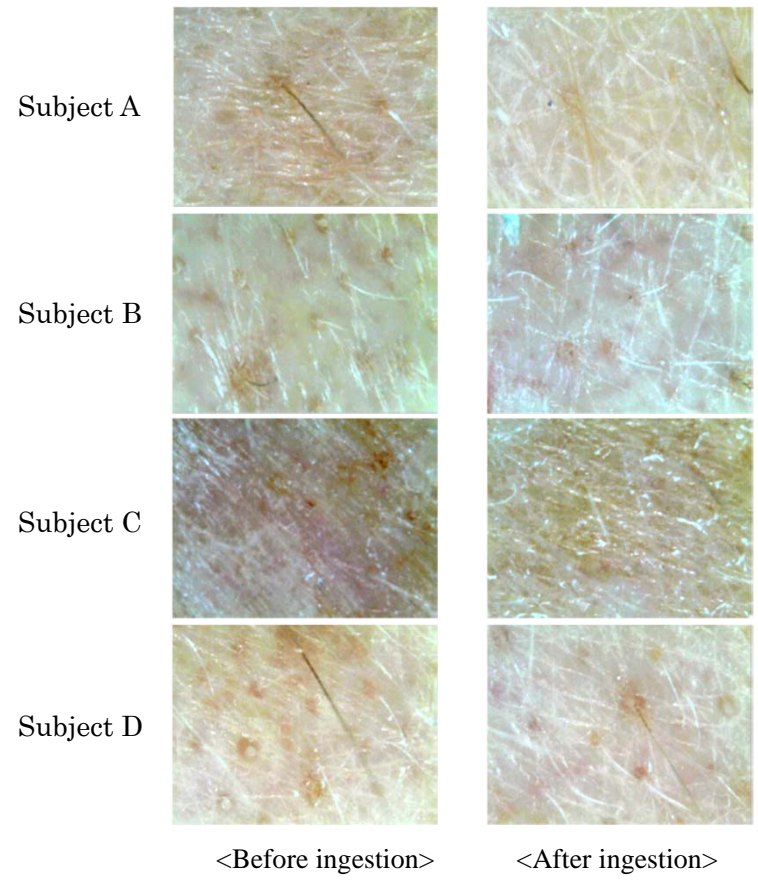


Fig.5 Subjects' feeling of skin condition improvement



**Fig.6 Microscopic images of skin (cheek):
the improved examples**

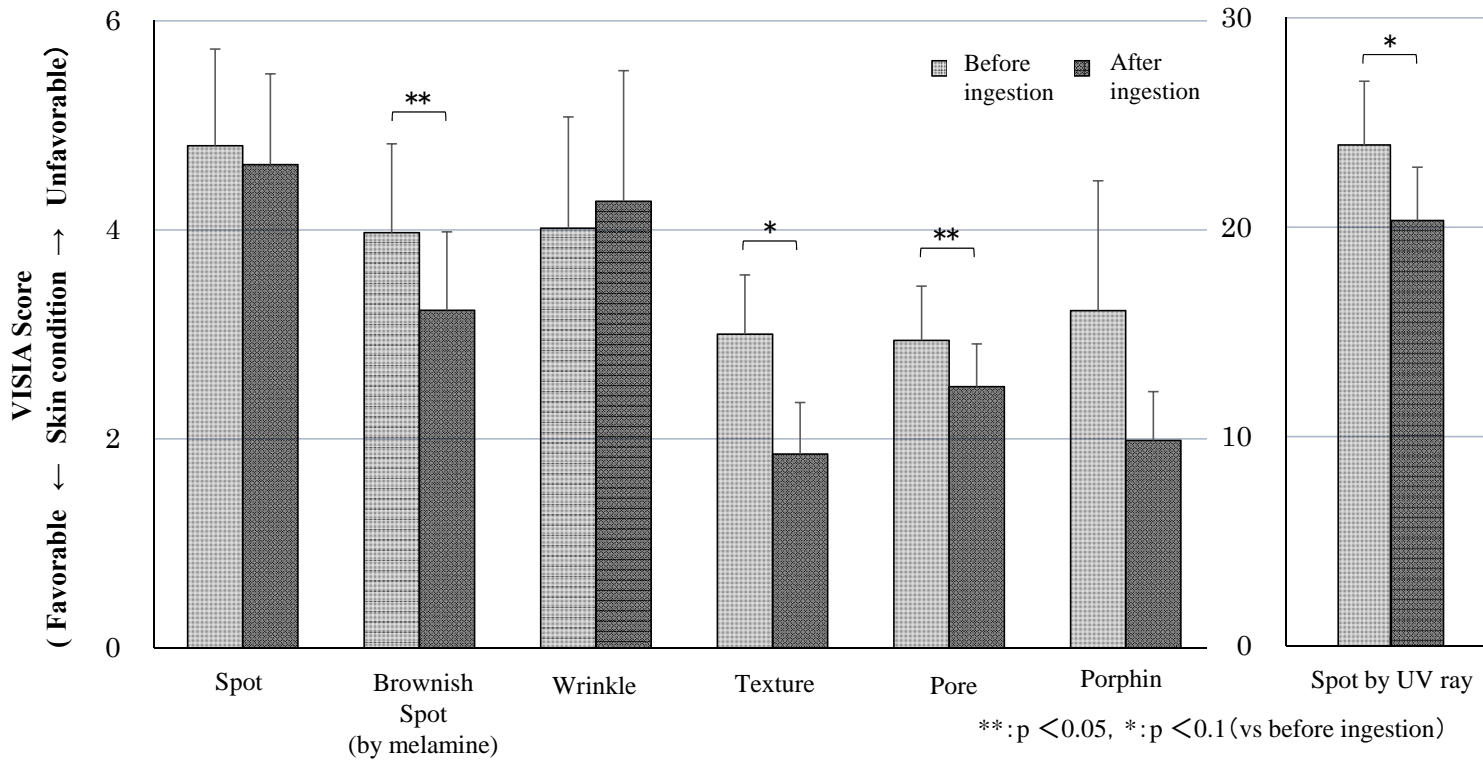


Fig.7 Skin condition change

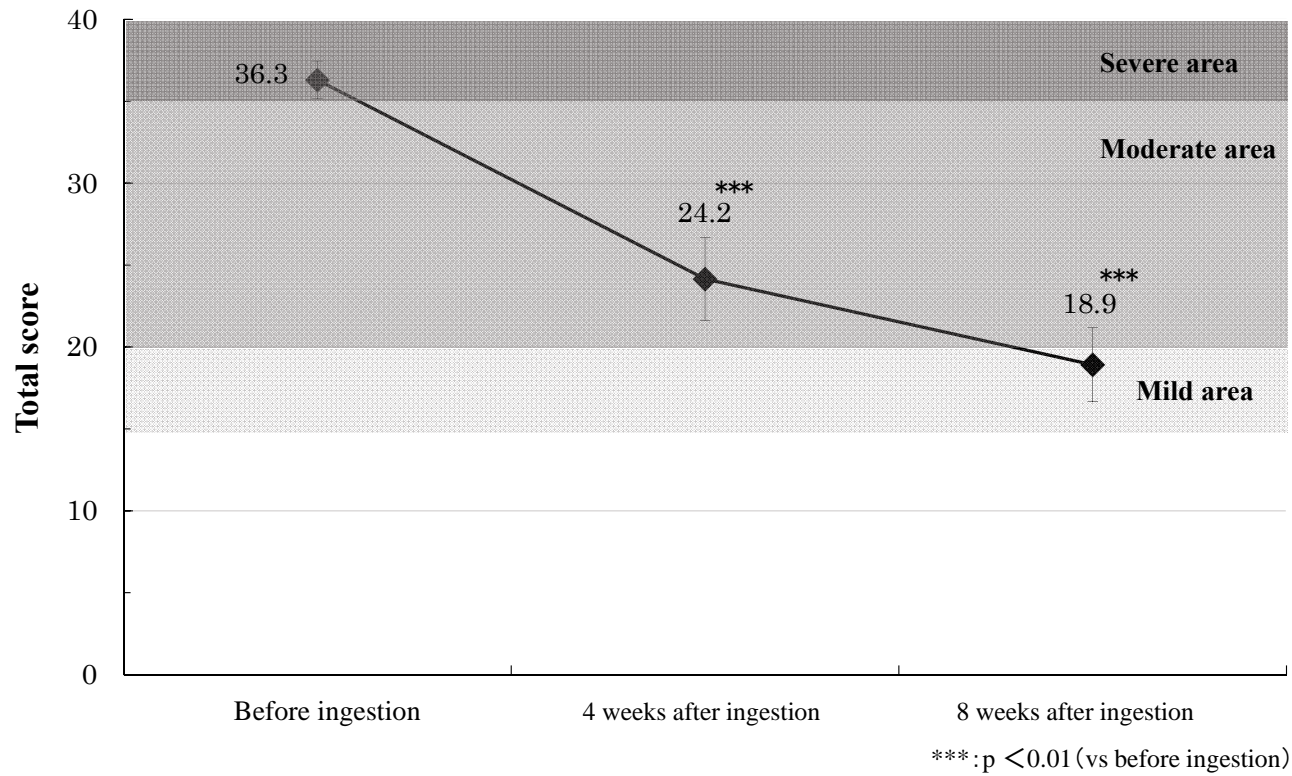


Fig. 8 Kuppermanmenopausalindex
/ Total score change of medical interview sheet

**Table 1: Kuppermanmenopausalindex
/ Total score change of medical interview sheet**

Item	Before ingestion	4 weeks after ingestion	8 weeks after ingestion
Vasomotor nerve disturbance-like symptom (glow/sweating/coldness/dyspnea)	10.5±2.0	8.00±4.00**	6.15±3.51***
Sensory disturbance-like symptom (limbs numbness)	2.92±1.93	1.69±1.80**	0.769±1.739***
Asomnia	5.38±0.96	3.23±2.24**	2.77±1.54***
Nervousness	4.77±1.30	3.08±1.55**	2.92±1.75**
Depression	2.46±0.78	1.54±1.13***	1.31±1.25***
Dizziness	1.00±0.58	0.385±0.506***	0.231±0.439***
General malaise	2.77±0.60	1.85±0.80***	1.62±0.77***
Arthralgia/ muscle soreness	2.62±0.51	1.92±1.04**	1.62±1.04**
Headache	1.85±0.80	1.54±1.20	1.08±1.12**
Rapid pulse	0.923±0.494	0.538±0.877	0.308±0.630***
Formication	1.15±1.07	0.385±0.650**	0.154±0.555**
Total	36.3±4.2	24.2±9.1***	18.9±8.1***

***:p <0.01, **:p <0.05 (vs before ingestion)

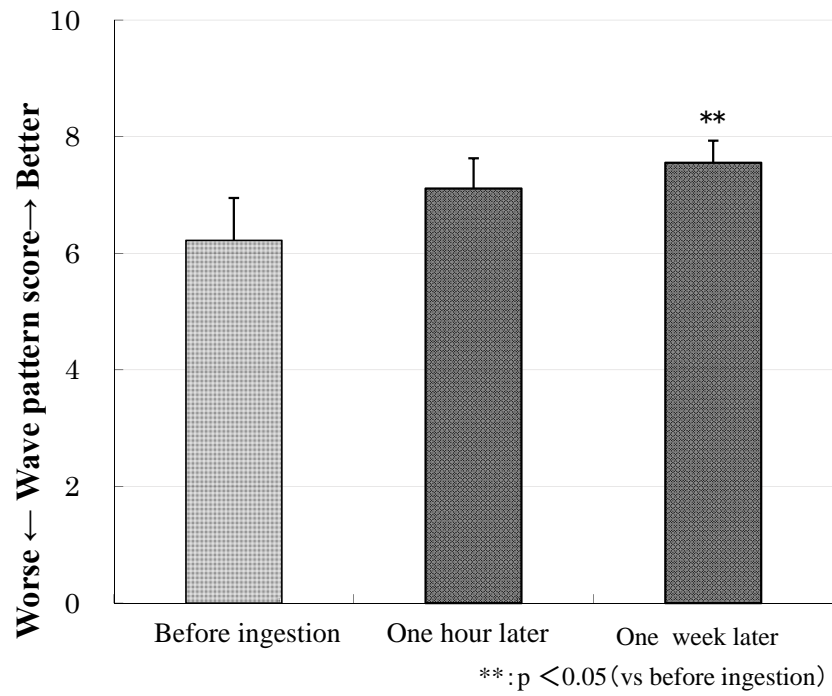


Fig.9 Change of peripheral blood circulation

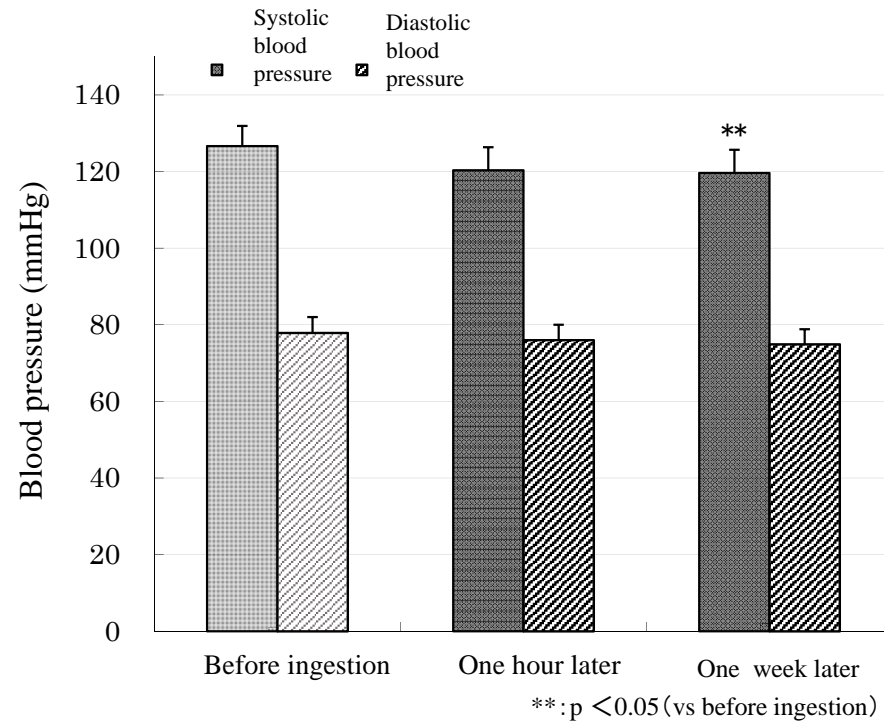


Fig.10 Change of blood pressure

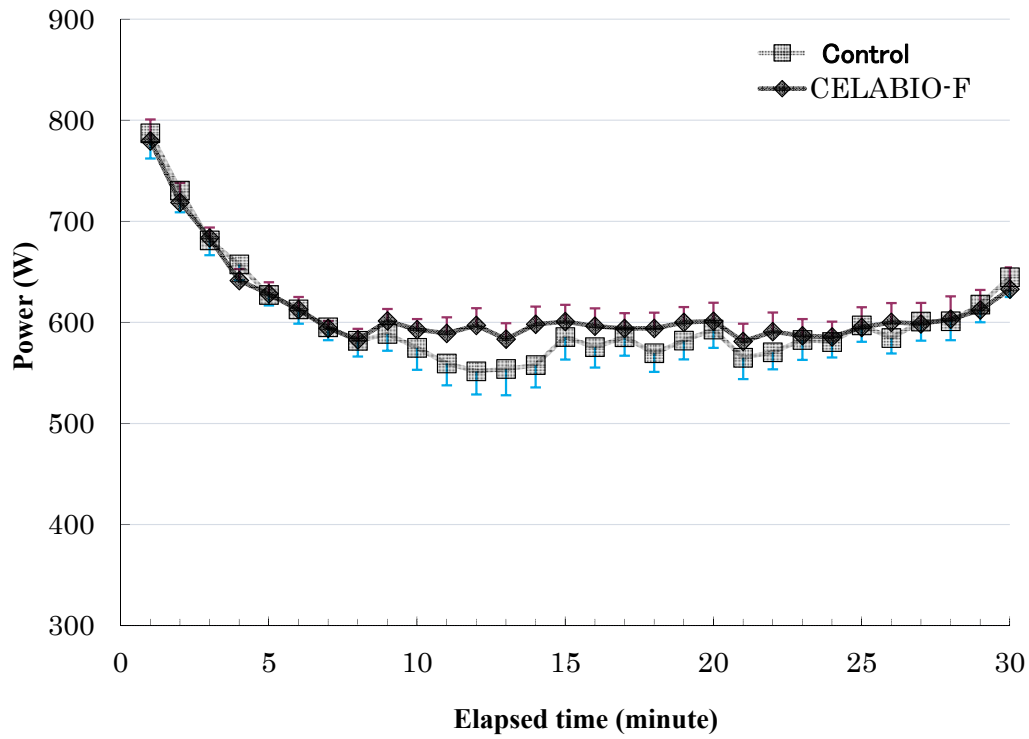


Fig.11 Exercise performance change

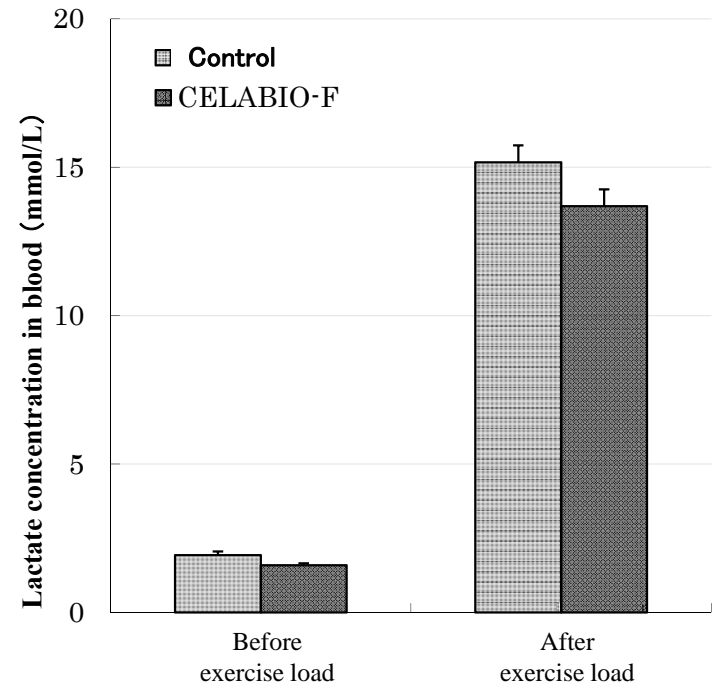


Fig.12 Change of lactate concentration in blood

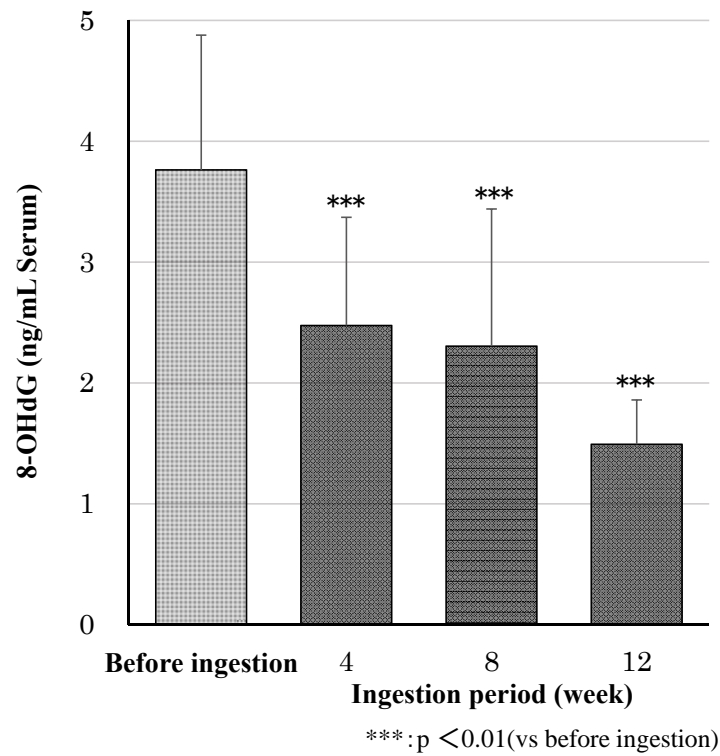


Fig. 13 Change of 8-OHdG concentration in blood

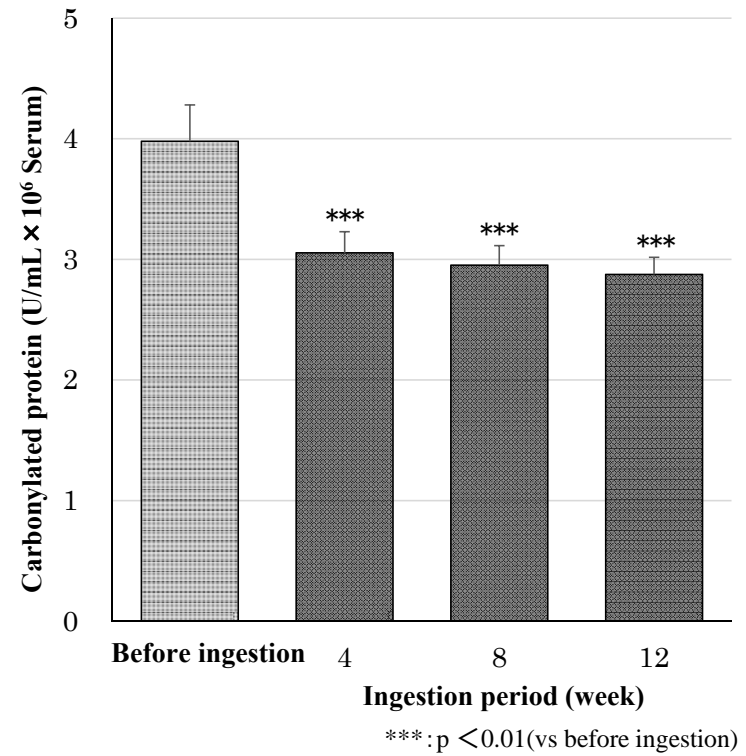


Fig.14 Change of carbonylated protein concentration in blood

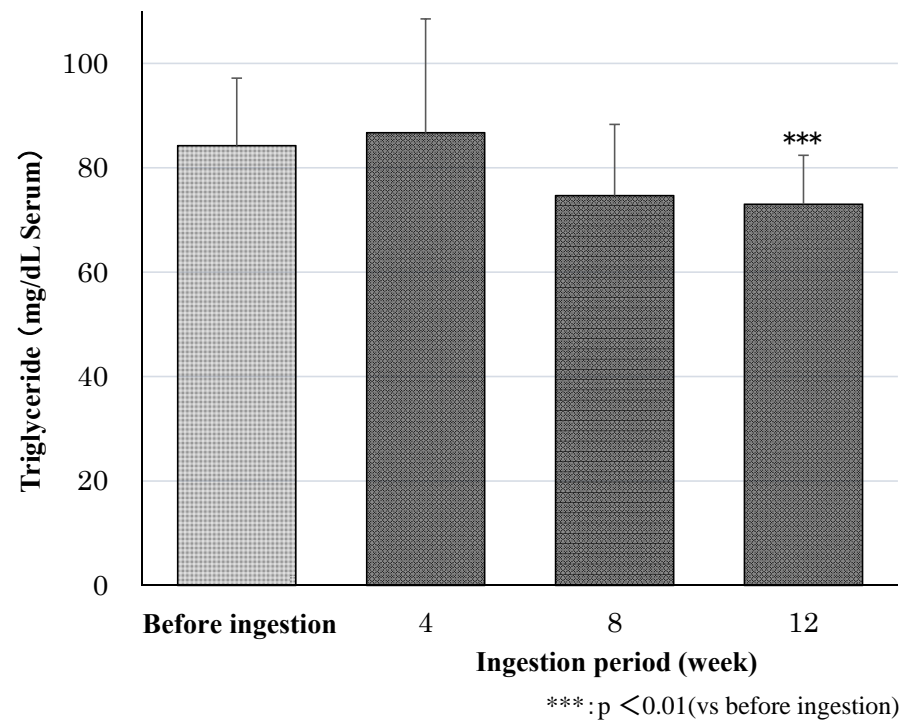


Fig.15 Change of triglyceride concentration in blood

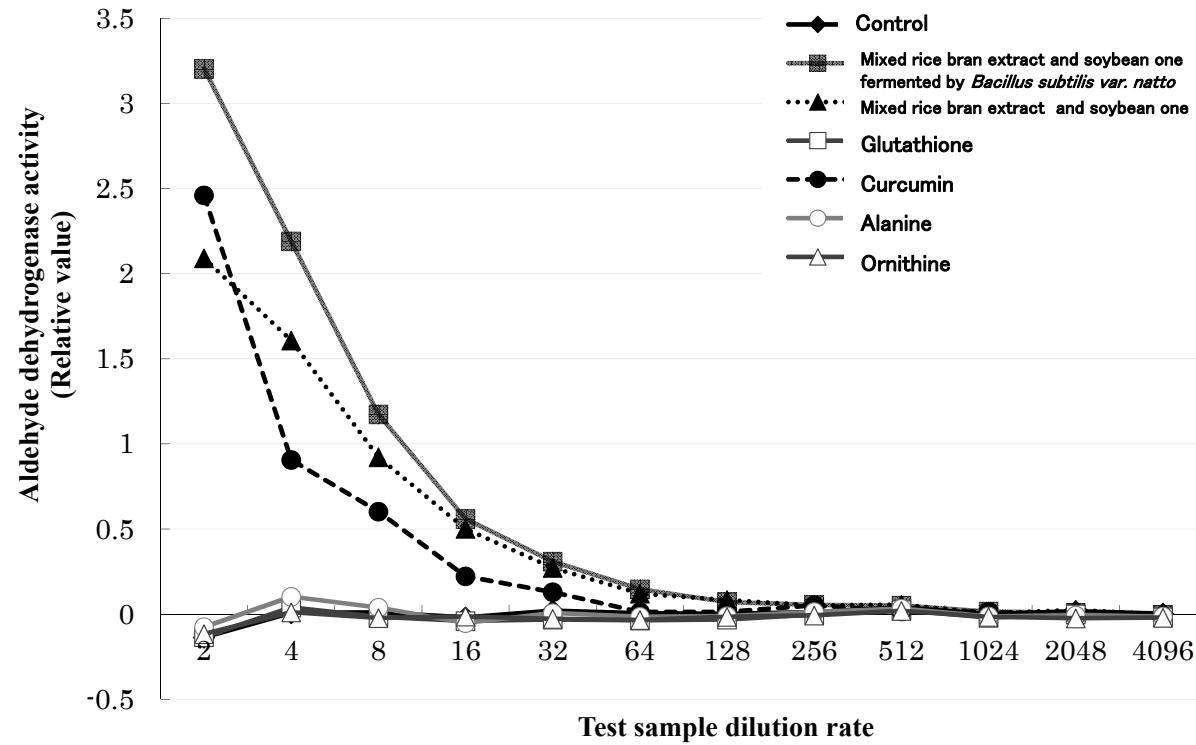


Fig.16 Aldehyde dehydrogenase activity in Chang Liver cell

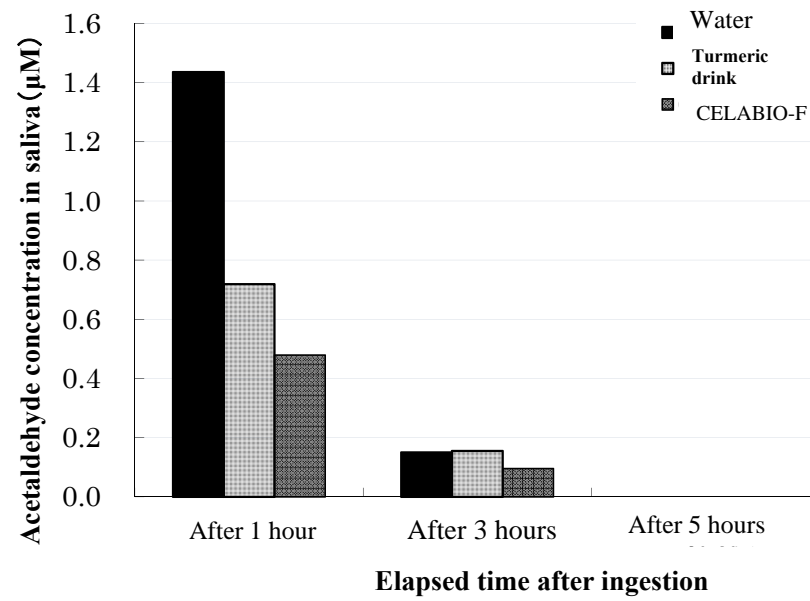


Fig. 17 Acetaldehyde metabolism acceleration

Table 2 Grouping

Group	Tested material	n
Control	Water	12
Group A	「CELABIO®-F」0.5g	13
Group B	Curcumin 30mg+「CELABIO®-F」0.5g	7
Group C	Japanese raisin tree extract	6
Group D	Silybum marianum extract	6
Group E	Mixed extract of rice bran extract and soybean extract	5

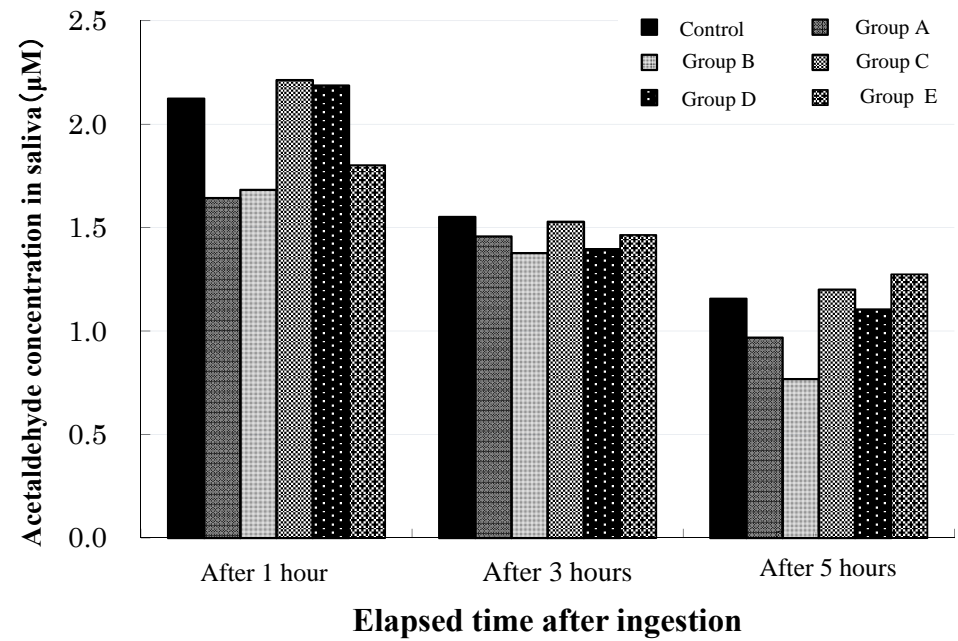


Fig.18 Accelerative effect on acetaldehyde metabolism