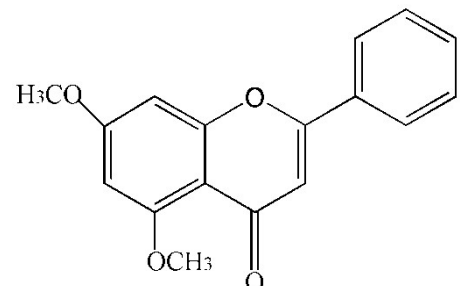


BLACK GINGER EXTRACT

Dietary ingredient for improvement of cold hands and feet and swelling, tonics, aphrodisiac, anti-obesity, physical fitness, muscular endurance and anti-fatigue

- **BLACK GINGER EXTRACT-P**
(Powder, Food Grade)
- **BLACK GINGER EXTRACT-WSP**
(Water-soluble Powder, Food Grade)
- **BLACK GINGER EXTRACT-PC**
(Powder, Cosmetic Grade)
- **BLACK GINGER EXTRACT-WSPC**
(Water-soluble Powder, Cosmetic Grade)
- **BLACK GINGER EXTRACT-LC**
(Liquid, Cosmetic Grade)



BLACK GINGER EXTRACT

Dietary ingredient for improvement of cold hands and feet and swelling, tonics, aphrodisiac, anti-obesity, physical fitness, muscular endurance and anti-fatigue

1. Introduction

[What is Black Ginger]

Black Ginger, a plant of the genus *Zingiberaceae Kaempferia*, commonly grows in the tropical Asia. Scientifically, it is known as *Kaempferia parviflora*, in Japan it is commonly referred as “black turmeric” or “black ginger”. Meanwhile, it is known as *Krachai Dam* in its country of origin, Thailand. Traditionally, Black Ginger is known as an energy enhancer with excellent tonic effect.



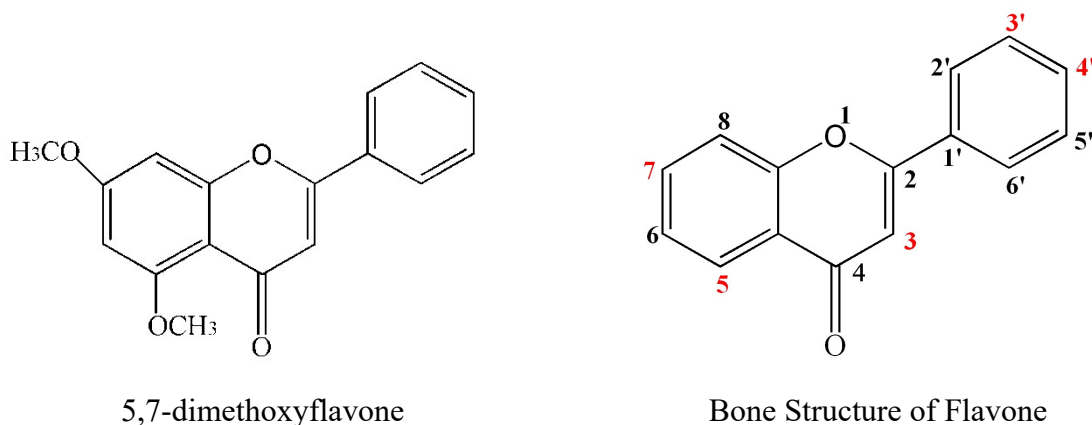
Fig. 1 Rhizome of Black Ginger, above the ground & under the ground

[Food uses of Black Ginger]

In South East Asia region, especially in Thailand, people drink tea boiled from sliced black ginger as well as alcohol soaked with black ginger. Alternatively, black ginger is commonly used a folk medicine for energy enhancement, and relief of gastrointestinal complaints. In conjunction with the “one village one product” campaign raised by the Thailand government, black ginger has been promoted as healthcare food to increase awareness among the public.

2. Functional Components of Black Ginger Extract

Black Ginger is loaded with flavonoids particularly with high content of polymethoxyflavone among all the flavonoids. In collaboration with Kyoto Pharmaceutical University, 8 variance of polymethoxyflavone were identified in Black Ginger Extract with the highest content of 5,7-dimethoxyflavone present. (Fig. 2)



Identified Constituents	substitutional groups				
	3	5	7	3'	4'
5-hydroxy-7-methoxyflavone		OH	OMe		
5,7-dimethoxyflavone (Specification compound)		OMe	OMe		
5-hydroxy-3,7-dimethoxyflavone	OMe	OH	OMe		
4',5,7-trimethoxyflavone		OMe	OMe		OMe
5-hydroxy-3,7,4'-trimethoxyflavone	OMe	OH	OMe		OMe
3',4',5,7-tetramethoxyflavone		OMe	OMe	OMe	OMe
5-hydroxy-3,7,3',4' -tetramethoxyflavone	OMe	OH	OMe	OMe	OMe
3,5,7,3',4' -pentamethoxyflavone	OMe	OMe	OMe	OMe	OMe

Fig. 2 Chemical structures of polymethoxyflavones in Black Ginger Extract.

Oryza Oil & Fat Chemical Co., Ltd. with its very own cutting edge technology in the extraction and purification of unique natural products, successfully developed Black Ginger Extract, a Thai origin raw material. Findings from human clinical trials reported that Black Ginger Extract improves peripheral blood circulation and peripheral vasculature. Black Ginger Extract, is a functional food ingredients with blood circulation enhancing effect and relief of edema.

In this brochure, we shall introduce various health promoting effect of Black Ginger Extract such as anti-inflammatory effect, aphrodisiac effect, prevention of metabolic syndrome (anti-obesity and anti-diabetes) and etc.

3. Functional Effects of Black Ginger Extract

(1) Improve Peripheral Blood Circulation and Relieves Edema

Cold extremities (or Raynaud’s phenomenon) is a condition where the hands and feet is feeling cold due to poor blood circulation to the extremities. Statistically, there is 1 in every 2 women and 1 in every 4 men is suffering from cold extremities.

Healthy blood circulation is important in the maintenance of homeostatic condition of our body (e.g. temperature and pH). Poor blood circulation to the extremities result in a lower temperature of the limbs, condition may worsen when outside temperature is lowered and vasoconstriction further reduced capillary circulation and oxygen supply to extremities causing painful sensation.

Edema is a swelling condition due to accumulation of interstitial fluid underneath the skin. Changes in the water retaining properties of tissues themselves, excessive intake of salt in the diet are major causes of edema. Other miscellaneous factors include remain sitting or standing in the same position for long time may contribute to edema.

Based on the above, improving peripheral blood circulation and peripheral vasculature is essential in the relief of cold extremities and edema.

1) Human Clinical Trial

A human clinical trial was conducted to examine the effect of Black Ginger Extract on peripheral blood circulation. In the trial, Black Ginger Extract-P (150mg/day) was given to 14 test subjects *ad libitum* in single dose and continuous intake for 1 week. Peripheral blood circulation and peripheral vasculature (shape and arrangement of blood vessels) as well as blood pressure was monitored before and after ingestion of Black Ginger Extract-P.

Trial Protocol:

- Test subjects: 8 healthy males (aged 24-59), 6 healthy females (aged 26-48)
- Test sample: Black Ginger Extract-P (contains >2.5% of 5,7-dimethoxyflavone; >10% of total flavonoids)
- Dosage: 150mg/day
- Test duration: Single dose and 1 week continuous intake
- Analysis parameter: Blood pressure (Terumo Digital BP Monitor)
Peripheral Blood Circulation (Blood Circulation checker)
Peripheral Vasculature (Blood Vessel Monitor)



A Blood Circulation Checker



A Blood Vessel Monitor

As shown in Fig. 3 and Table. 1, peripheral blood circulation of test subjects improved 1 hour after oral administration of Black Ginger Extract-P 150mg. In addition, Table 2 illustrated that peripheral blood circulation improved 57.1% and 50.0% after 1 hour and 1 week continuous intake of Black Ginger Extract-P 150mg respectively.

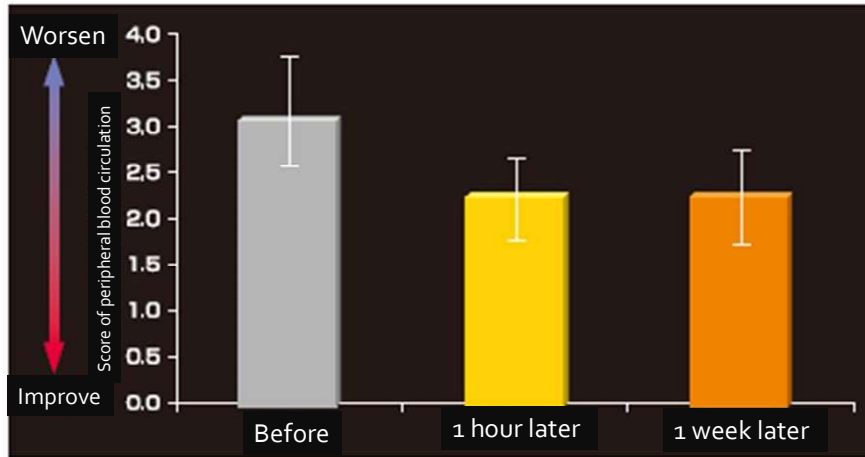


Fig. 3 Effect of Black Ginger Extract-P on Peripheral Blood Circulation (n=14, Mean ± SE)

Table 1. Readings of Peripheral Blood Flow condition

Result	A+	A	A-	B+	B+X	B	BX	C+	C	B-	B-X	C-	E+	E	D+	D	D-	E-	F/F-	G/G-
Digitalisation	0	0.5	1	1.5	2.5	3	3.5	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5
Assessment	Excellent condition ←										→ Low condition									

Table 2. Improvement of Peripheral Blood Flow (Red indicated improvement against initial, refer to table 1, n=14)

Test Subjects	Results		
	Initial	1 hour later	1 week later
A	C	C+	B
B	B-	B+X	B+
C	B	B+	B+
D	A-	C+	B
E	B+X	B+X	BX
F	B-	B+	B
G	C+	A-	A-
H	B+	A	A
I	C-	B-	E
J	A	B+X	A
K	A	A-	A-
L	C	A-	B+
M	B+	B+	B+X
N	A-	A-	A-
Improvement rate (%)	-	57.1	50.0

Meanwhile, peripheral vasculature (disposition and arrangement of blood vessels) was observed to return to its normal arrangement from a deformed state. (Fig. 4).



Fig. 4 Effect of Black Ginger Extract-P on Peripheral Vasculature

Furthermore, both systolic and diastolic blood pressure of test subjects was regulated to normal range 1 hour after single dose oral administration of Black Ginger Extract-P 150mg (Table 3, Fig. 5). Systolic BP was significantly reduced 2 hours after the single dose oral administration ($P < 0.05$) and the blood pressure regulation effect was maintained for 2 hours.

Table 3. Effect of Black Ginger Extract-P on Blood Pressure (BP reading, n=14)

Subjects	Before		1H post administration		2H post administration		After 1W administration	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
Male								
A	117	69	124	70	115 ↓	65 ↓	106 ↓	75
B	149	101	141 ↓	90 ↓	145 ↓	97 ↓	144 ↓	98 ↓
C	101	62	109	70	108	60 ↓	109	74
D	137	102	133 ↓	90 ↓	120 ↓	94 ↓	130 ↓	98 ↓
E	121	63	112 ↓	63	106 ↓	63	112 ↓	66
F	126	96	120 ↓	83 ↓	121 ↓	91 ↓	120 ↓	88 ↓
G	107	72	119	65 ↓	113	74	110	69 ↓
H	133	80	125 ↓	83	116 ↓	78 ↓	132 ↓	87
Female								
I	133	82	149	94	155	92	-	-
J	99	66	97 ↓	67	95 ↓	62 ↓	109	68
K	126	57	113 ↓	63 ↓	122 ↓	65	128	76
L	135	81	97 ↓	76 ↓	109 ↓	85	116 ↓	66 ↓
M	117	73	105 ↓	63 ↓	101 ↓	58 ↓	104 ↓	73
N	105	71	106	68	107	73	104	68
Average	121.9	76.8	117.9	74.6	116.6	75.5	117.2	77.4
SE	4.0	3.8	4.2	3.0	4.3	3.7	3.4	3.1
Improvement Rate (%)			57.1	50.0	71.4	57.1	61.5	38.5

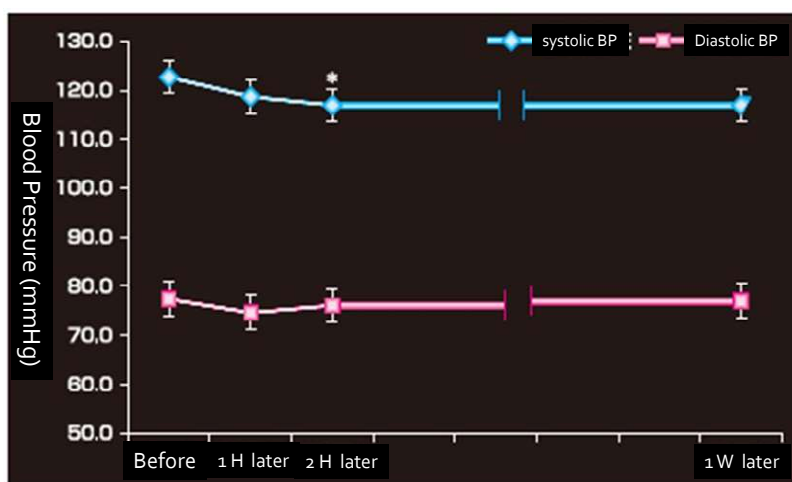


Fig. 5 Effect of Black Ginger Extract-P on Blood Pressure (n=13~14, Mean ± SE, *: $P < 0.05$)

Questionnaire survey was conducted to evaluate the subjective comments of test subjects. Most responded that symptoms of edema, circulation, dry skin feeling and energy level noticeably improved (Fig. 6).

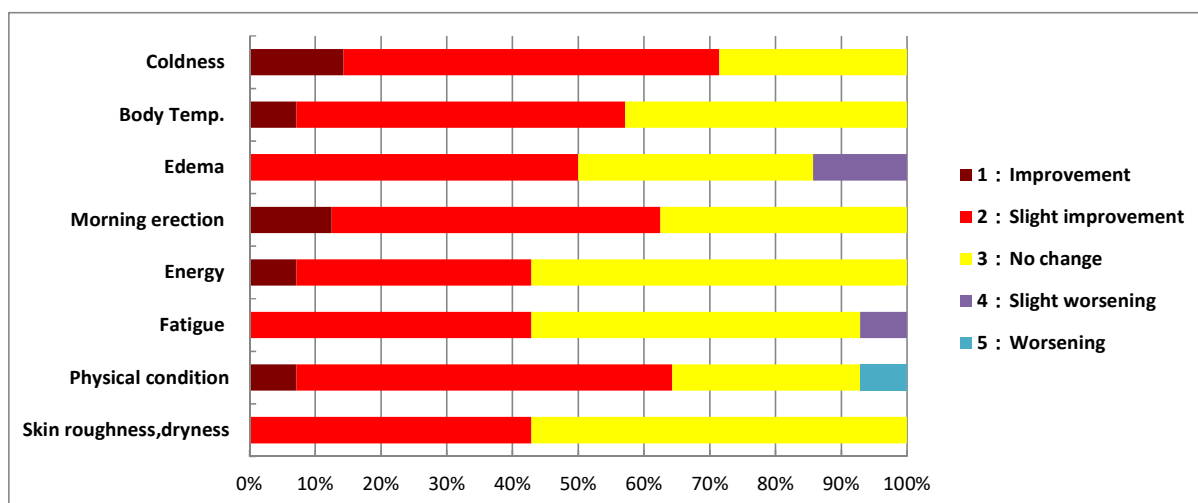


Fig.6 The Subjective comments on Black Ginger Extract-P

Above findings showed that continuous oral intake of Black Ginger Extract-P 150mg/day improved peripheral blood circulation, improve peripheral vasculature and relieved symptoms of edema.

In addition, blood pressure of test subjects was regulated to normal range after oral intake of Black Ginger Extract-P, in particular, systolic blood pressure significantly reduced and regulated to optimum range 2 hours after oral administration of Black Ginger Extract-P.

2) Promotion of Nitric Oxide (NO) Production in Endothelial Cells and Vascular Function

Nitric Oxide (NO) also known as “endothelium-derived relaxing factor”, synthesized endogeneously by activation of various nitric oxide synthase (NOS). NO function as cell signaling factors in physiological and pathological processes.

NOS is classified into 2 major groups: Constitutive NOS (cNOS) that present in the cells at all times and the other inducible NOS (iNOS) in response to inflammation and stress. Constitutive NOS (cNOS) includes endothelial constitutive (eNOS) and brain constitutive (nNOS). Neuronal NOS (nNOS) produces NO in nervous tissue of central and peripheral nervous system. Meanwhile, endothelial NOS (eNOS) generates NO in blood vessels and involved in regulating blood vessel function especially vasodilation.

In a study conducted by Wattanapitayakul *et al.*, reported that Black Ginger Extract enhanced nitric oxide (NO) production in human umbilical vein endothelial cells (Fig. 8) and eNOS mRNA and protein expression were up-regulated (Fig. 7).

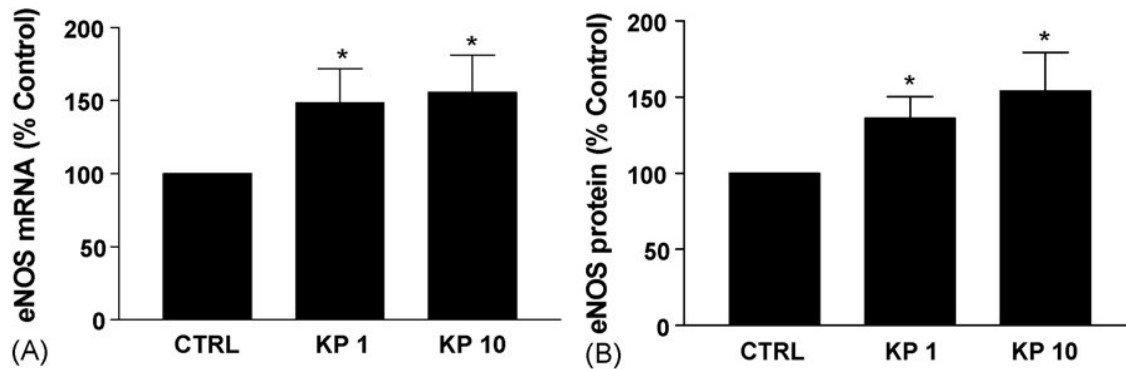


Fig. 7 The Effect of Black Ginger Extract on eNOS mRNA expression and eNOS protein expression. CTRL: Control, A: eNOS mRNA genetic expression, B: eNOS protein expression, KP1: Black Ginger Extract 1µg/mL, KP10: Black Ginger Extract 10µg/mL. Data are Mean ± SE, *: $P < 0.05$ vs. control.

In addition, the effect of Black Ginger Extract at 10µg/mL on NO production was stronger than acetylcholine, the positive control (1000µg/mL) (Fig. 8). Meanwhile, production of NO was not affected by L-NAME, inhibitor of NO production, in samples containing Black Ginger Extract.

Endothelial NOS facilitate vascular function by generating NO production in the blood vessels, inhibit smooth muscle contraction and platelet aggregation. Black Ginger Extract enhances NO production, therefore, improve blood circulation and regulate blood pressure. It is potentially beneficial in vascular endothelial health promotion such as prevention of arteriosclerosis.

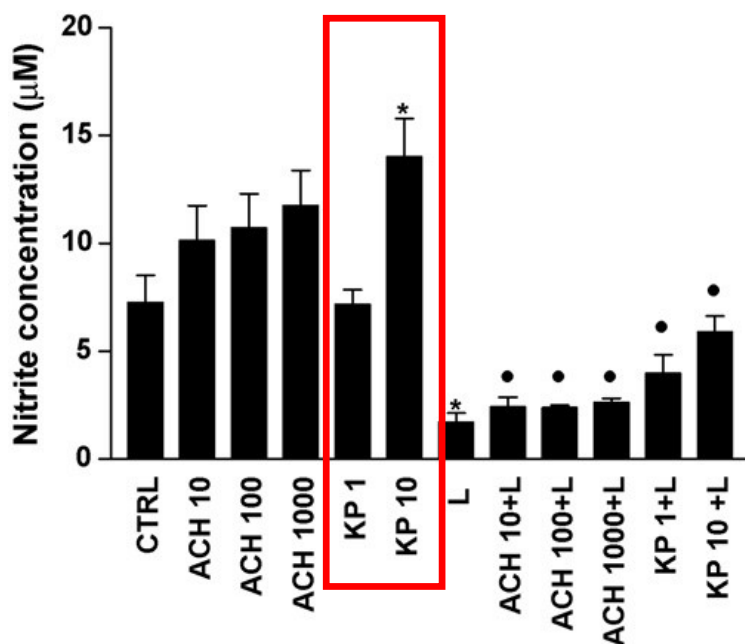


Fig. 8 The Effect of Black Ginger Extract on NO production in human umbilical vein endothelial cells. CTRL: Control, ACH: acetylcholine, NO production inducer, positive control, KP1, KP10: Black Ginger Extract 1, 10µg/mL, L: L-NAME, NO production inhibitor, negative control. Data are Mean \pm SE, *: $P < 0.05$ vs. control, •: $P < 0.05$ vs corresponding treatments without L-NAME.

Reference:

S.K. Wattanapitayakul *et al.* *Journal of Ethnopharmacology* **110**, 559–562 (2007)

(2) Improvement of physical fitness and muscular endurance

1) Enhancement of muscular metabolisms *in vitro*

AMP-activated protein kinase (AMPK) is known to be critically involved in the regulation of energy homeostasis, and its activation has been shown to enhance the metabolism of glucose and lipids. Therefore, AMPK has been an attracting target for the discovery of anti-diabetic or anti-obesity treatments. In addition, phosphorylation of AMPK is linked to physical activity and muscular endurance. 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), an agonist of AMPK, was previously reported to increase running endurance by up to 44% and decrease body fat in mice when orally administered for 4 weeks. Consequently, the phosphorylation of AMPK has been suggested to improve physical fitness performance, muscular endurance, and fat metabolism.

We reported polymethoxyflavones (PMF) in black ginger extract enhanced muscular metabolism through phosphorylated AMPK in C2C12 myoblast. In addition, BLACK GINGER EXTRACT improved consumptions of glucose, lipid and lactic acid, mitochondrial number, accumulation of muscular glycogens, and muscular inflammation in C2C12.

Therefore, parts of PMF in BLACK GINGER EXTRACT, activated AMPK, was expected improvement of not only physical fitness but also obesity and hyperglycemia.

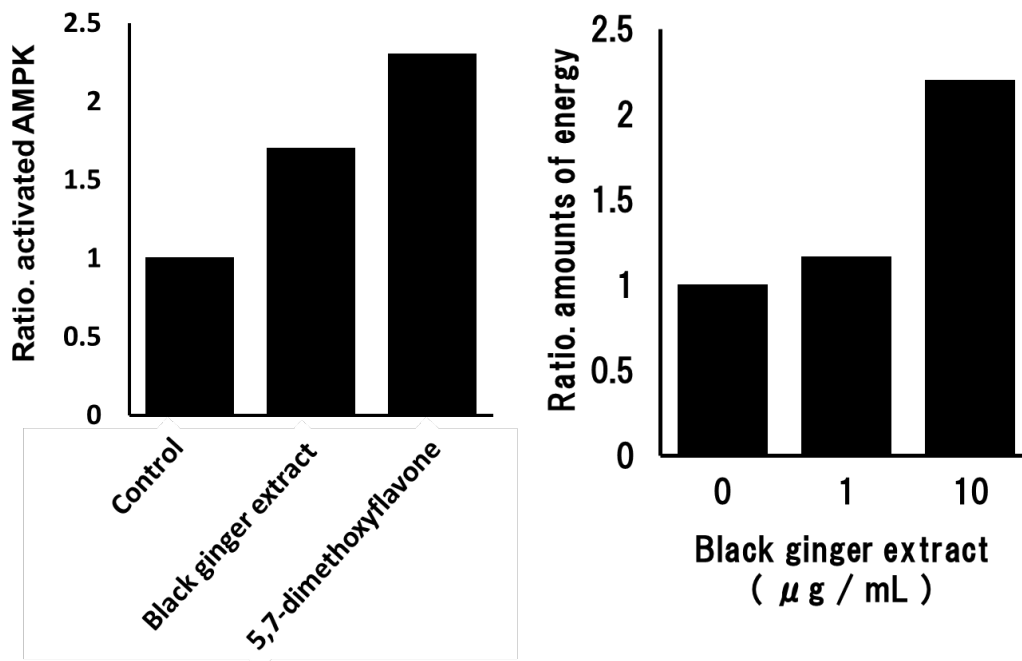
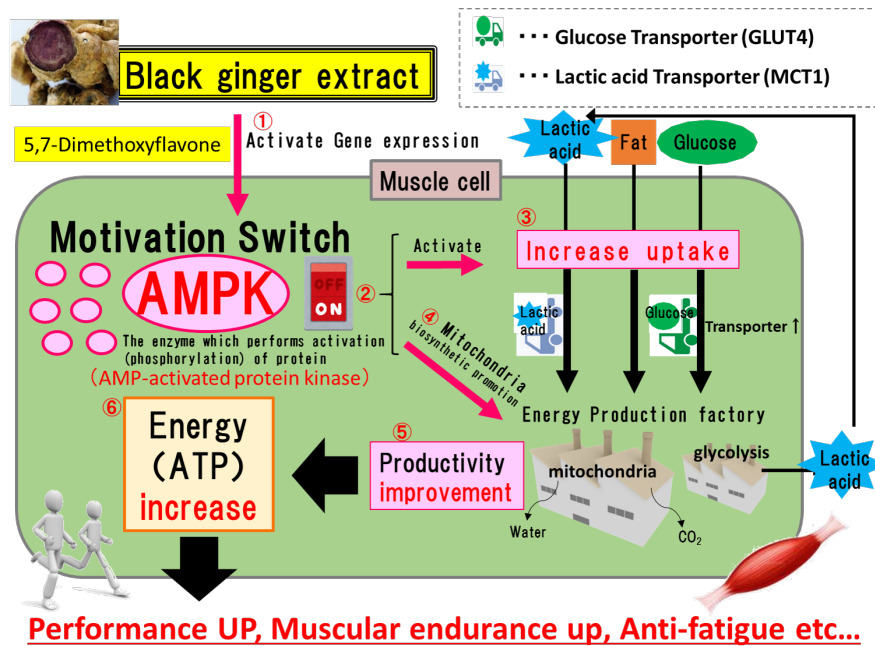


Fig. 9 The Effect of black ginger extract and 5,7-dimethoxyflavone on activation of AMPK (left) and energy productions (right) in C2C12 myoblast.

Reference:

- * Kahn, B.B., Alquier, T., Carling, D., Hardie, D.G. 2005. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metabolism* 1, 15-25
- * Hardie, D.G., Schaffer, B.E., Brunet, A. 2016. AMPK: an energy-sensing pathway with multiple inputs and outputs. *Trends in Cell Biology* 26, 190-201
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- * Toda, K., Takeda, S., Hitoe, S., Nakamura, S., Matsuda, H., Shimoda, H., 2016. Enhancement of energy production by black ginger extract containing polymethoxy flavonoids in myocytes through improving glucose, lactic acid and lipid metabolism. *Journal of Natural Medicines* 70, 163-172.
- * Toda, K., Hitoe, S., Takeda, S., Shimoda, H., 2016. Black ginger extract increases physical fitness performance and muscular endurance by improving inflammation and energy metabolism. *Heliyon* 2 (2016) e00115

2) Increase in physical fitness and muscular endurance *in vivo*

Previously, we evaluated effect of BLACK GINGER EXTRACT, activated AMPK in C2C12 myoblast, on improvement of physical fitness performance and muscular endurance in male ddY mice aged 10 weeks old. The BLACK GINGER EXTRACT group was orally administered BLACK GINGER EXTRACT (45 mg/kg/day) suspended in water for 4 weeks. And forced swimming test, open-field test, inclined plane test, and wire hanging test were performed at 0, 1, 2 and 4 weeks. As a result, BLACK GINGER EXTRACT increases muscular endurance (Fig. 10 and 11) and physical fitness performance (Fig. 11) in above tests.

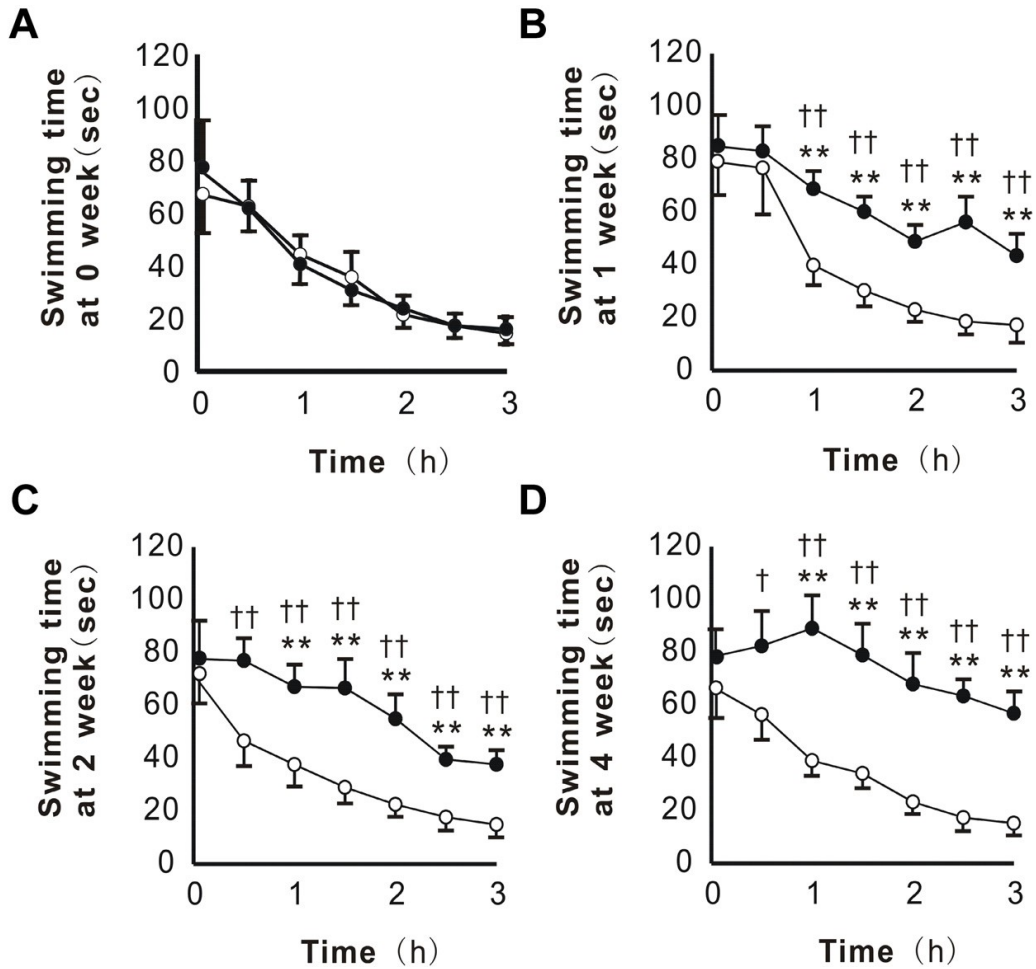


Fig. 10 BLACK GINGER EXTRACT enhanced muscular endurance in the consecutive forced swimming test (CST).

The CST was performed at the 0-(A), 1-(B), 2-(C), and 4-(D) week periods. ST was repeated at 30-min intervals, and the swimming time was measured for a total of 7 times. Each point represents the mean with the S.E. (control; n=15, BLACK GINGER EXTRACT; n=14). Open circle (○) for the control group and closed circle (●) for the BLACK GINGER EXTRACT group. Asterisks denote significant differences from the initial value (0 week) at *: P<0.05, **: P<0.01, respectively. Daggers denote significant differences from the control at †: P<0.05, ††: P<0.01, respectively.

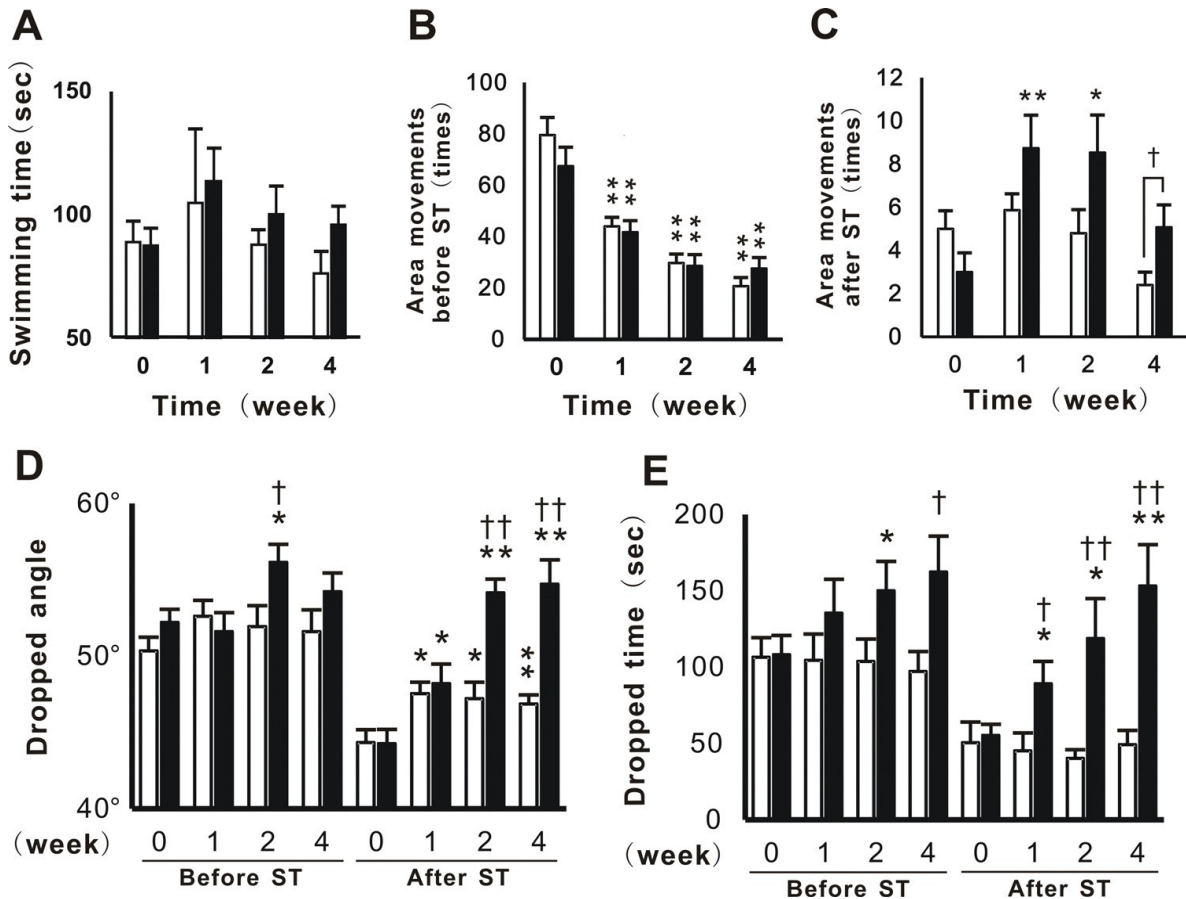


Fig. 11 BLACK GINGER EXTRACT enhanced physical fitness performance with or without fatigue loading.

Physical fitness measurement tests (PT) consisting of forced swimming test (ST, A), open-field test (B and C), inclined plate test (D) and wire hanging test (E) were performed. Columns represented open column (□): control group and closed column (■): BLACK GINGER EXTRACT group. Open-field test, inclined plate test, and wire hanging test were performed twice: before and after ST. The values in the open-field test before (B) and after (C) ST were indicated separately. Each column represents the mean with the S.E. (control; n=15, BLACK GINGER EXTRACT; n=14). Asterisks denote significant differences from the initial result (0 week) at *: P<0.05, **: P<0.01, respectively. Daggers denote significant differences from the control at †: P<0.05, ††: P<0.01, respectively.

Reference:

Toda, K., Hito, S., Takeda, S., Shimoda, H., 2016. Black ginger extract increases physical fitness performance and muscular endurance by improving inflammation and energy metabolism. *Heliyon* 2 (2016) e00115

3) Clinical trial

Previous clinical studies were indicated that BLACK GINGER EXTRACT might possess the ability to enhance physical fitness, namely, grip strength, leg muscle strength, balance, endurance, and locomotor activity in athletes, elder people and healthy people.

Our company evaluated the effect of BLACK GINGER EXTRACT on health-related quality physical fitness in the healthy volunteers who are aged from 20 to 62. This study was performed as a randomized double-blind placebo controlled crossover trial. 24 healthy volunteers were recruited. Each volunteer took one capsule containing BLACK GINGER EXTRACT - P (100 mg/day) or the placebo once a day for 4 weeks.

In order to evaluate the effects of BLACK GINGER EXTRACT on physical fitness and endurance, a physical fitness test (PT) consisting of a hand grip strength test, 30-second chair stand test, 5-m tandem walking test, and cycle ergometer test, was performed. The grip strength after a 4-week treatment with BLACK GINGER EXTRACT significantly increased (right: +2.2 kg, $P < 0.05$, left: +2.8 kg, $P < 0.01$) from baseline. Significant improvements were also observed in the 30-second chair stand test (+6.3 times, $P < 0.01$), 5-m tandem walking test (-3.2 sec, $P < 0.05$), and cycle ergometer test (+8.6 kcal, $P < 0.01$). The grip strength of right hand was significantly higher after the ingestion of BLACK GINGER EXTRACT (44.6 kg, $P < 0.05$) than after that of the placebo (43.0 kg).

Net changes in the grip strength of left hand (+2.8 vs +0.0 kg, $P < 0.05$), 30-second chair stand test (+6.3 vs +1.7 times, $P < 0.05$), 5-m tandem walking test (-3.2 vs -0.9 sec, $P < 0.01$), and cycle ergometer test (+8.5 vs +1.1 kcal, $P < 0.01$) were significantly greater after the intake of BLACK GINGER EXTRACT than the values after the placebo ingestion. These results indicate that BLACK GINGER EXTRACT possesses the ability to enhance physical fitness, namely, grip strength, leg muscle strength, balance, endurance, and locomotor activity.

Table 4. Effects of BLACK GINGER EXTRACT on physical fitness and fatigue in healthy people

Measured parameters		Before	After	Net change (Δ)
Grip strength (R) (kg)	Placebo	42.8 \pm 2.5	43.0 \pm 2.5	0.21 \pm 0.7
	Black ginger extract	42.4 \pm 2.4	44.6 \pm 2.6 ^{*,†}	2.17 \pm 0.9
Grip strength (L) (kg)	Placebo	40.1 \pm 2.5	40.1 \pm 2.5	0.03 \pm 0.8
	Black ginger extract	38.9 \pm 2.2	41.7 \pm 2.2 ^{††}	2.80 \pm 0.8 [*]
30-second chair stand test (sec)	Placebo	25.3 \pm 2.1	27.0 \pm 1.8	1.71 \pm 0.9
	Black ginger extract	21.4 \pm 1.7	27.6 \pm 1.7 ^{††}	6.27 \pm 1.7 [*]
5-m tandem walking test (sec)	Placebo	12.2 \pm 0.8	11.4 \pm 0.9	-0.87 \pm 0.5
	Black ginger extract	13.8 \pm 1.3	10.6 \pm 0.9 [†]	-3.17 \pm 1.3 ^{**}
Cycle ergometer test (kcal)	Placebo	47.4 \pm 4.5	48.6 \pm 4.3	1.13 \pm 1.4
	Black ginger extract	44.3 \pm 4.0	52.9 \pm 4.7 ^{††}	8.54 \pm 1.9 ^{**}

Data were presented as the mean \pm S.E (n = 24). Significant differences from the placebo were indicated as ^{*}: P<0.05, ^{**}: P<0.01, and those from the baseline were indicated as [†]: P<0.05, ^{††}: P<0.01 (paired t-test).

Reference:

- * Promthep, K., Eungpinichpong, W., Sripanidkulchai, B., Chatchawan, U., 2015. Effect of *Kaempferia parviflora* extract on physical fitness of soccer players: a randomized double-blind placebo-controlled trial. Medical Science Monitor Basic Research 21, 100-108
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- * Kazuya, T., Marina, K., Shogo, T., Shoketsu, H., Norihito, S., Hiroshi, S. 2016. Enhancement of physical fitness by black ginger extract rich in polymethoxyflavones: a double-blind randomized crossover trial. Integrative Molecular Medicine 3, 628-634

(3) Aphrodisiac Effect

1) Inhibition of Phosphodiesterase-5

Phosphodiesterase or cyclic nucleotide phosphodiesterase consist of group of enzymes which degrades intracellular second messenger cGMP and cAMP. Phosphodiesterase type 5 (PDE 5), is one of the 11 enzymes that selectively degrades cGMP in vascular smooth muscle cells supplying the corpus cavernosum of the penis. Inhibition of PDE5 increases level of cGMP leading to smooth muscle relaxation, vasodilation and increased blood flow to the penile tissue. Therefore, PDE5 inhibitor is used in the treatment of erectile dysfunction (ED), e.g. Sildenafil of Viagra.

Temkitthawon *et al.*, conducted a study to evaluate the effect of Black Ginger Extract on phosphodiesterase-5. Result showed that Black Ginger Extract demonstrated inhibitory effect on PDE5 at concentration of 1µg/mL and 10µg/mL (Fig 12). Furthermore, 5,7-dimethoxyflavone, the principal functional component of Black Ginger Extract has similarly inhibited PDE5 at concentration of 30µM (Fig 12).

Similar to VIAGRA, a therapeutic agent for ED, Black Ginger Extract is aphrodisiac by inhibiting PDE5 activity resulting in smooth muscle relaxation, vasodilation and increased blood flow to penile tissue. Table 5 showed that 5,7 dimethoxyflavone, the principal component of Black Ginger Extract, is the most potent flavones in PDE5 inhibition. However, no effect PDE5 observed in samples treated with Piper Longum and Ginger Extract (Fig 12).

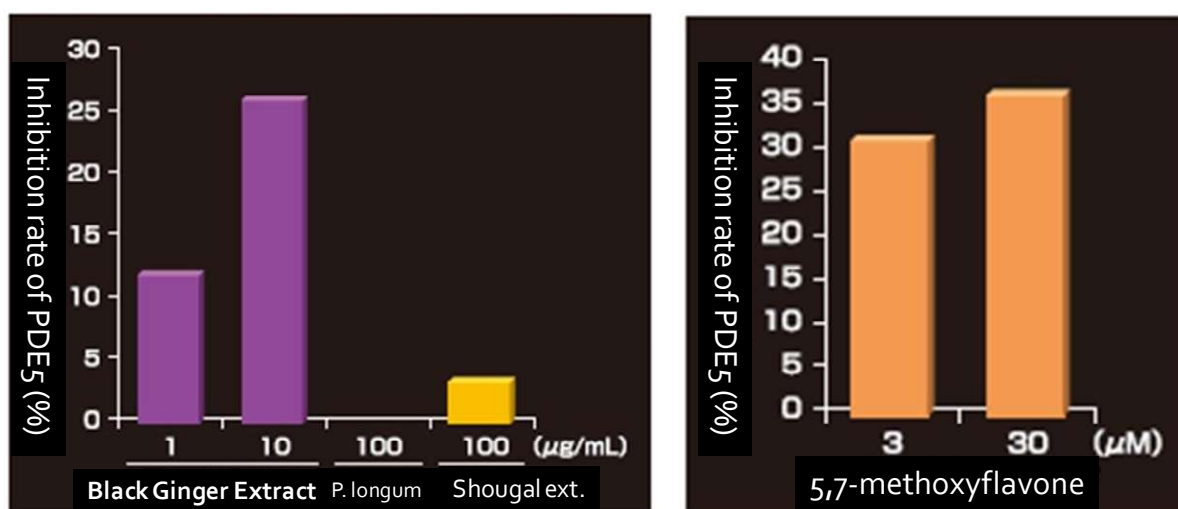


Fig. 12 The inhibitory effect of Black Ginger Extract and 5,7 dimethoxyflavone on PDE 5

Table 5. Inhibitory effect of functional components of Black Ginger Extract on PDE5

Compound	Inhibition rate on PDE5 (% , 10 µ M)
5-hydroxy-7-methoxyflavone	18.23 ± 3.26
5,7-dimethoxyflavone	53.65 ± 1.15
5-hydroxy-7,4'-dimethoxyflavone	17.64 ± 3.19
4',5,7-trimethoxyflavone	37.82 ± 4.08
5-hydroxy-3,7-dimethoxyflavone	0.76 ± 1.26
3,5,7-trimethoxyflavone	44.96 ± 2.43
5-hydroxy-3,7,4' -trimethoxyflavone	6.02 ± 5.94
3,5,7,3',4' -pentamethoxyflavone	37.55 ± 2.07

Reference: Modified from P. Temkitthawon *et al. Journal of Ethnopharmacology* **137**, 1437– 1441 (2011)

2) Increase in production of testosterone in testicular cells

Testosterone is well-known as one of the male hormones which affects promotion of sexual desire, development of sexual organs, hypertrophy skeletal muscle and so on... In addition, recent studies reported that the hormone was related to life span, brain function and metabolic syndromes. Therefore, testosterone is called as “regenerated hormone”.

In case of male, testosterone is mostly synthesized and secreted in the testes (testicles). Our company performed to evaluated production of testosterone *in vitro* test using R2C cells, testicular Leydig cells. In this assay, black ginger extract (Black ginger; B), maca extract (Maca; M), 5,7-dimethoxyflavome (DMF) and benzyglucosinolate (BG) increased the production of testosterone compared with control. Interestingly, the combining black ginger with maca was suggested additive effect.

Amounts of intracellular testosterone (Testicle)

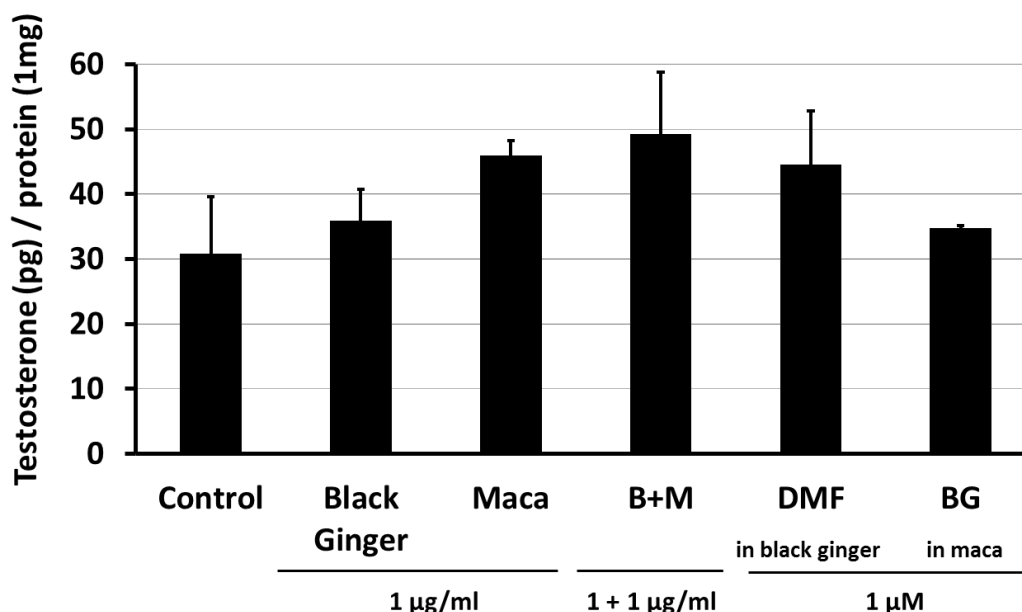


Fig. 13 The additive effect of black ginger and maca on production of testosterone

(4) Anti-metabolic Syndrome (anti-obesity, anti-diabetic)

It has been estimated that there is approximately 86 million people suffering from metabolic syndrome in 6 most industrialized countries in the world. Recently, there are increasing cases of obesity in Japan due to irregular physical activity and dietary habits. The

Ministry of Health, Labor and Welfare reported that about 19 million Japanese in age group of 40-74 years were estimated to have metabolic syndrome, i.e. 1 in 2 males and 1 in 5 females are affected. In addition, sedentary lifestyle has been a huge consequence of metabolic syndrome and diseases such as hypertension, hyperlipidemia, diabetes and obesity. As a result, metabolism is disrupted with increasing visceral fat accumulation, insulin resistance and high blood cholesterol level. Besides, the equilibrium of physiologically active substances such as adipokines secretion is disrupted.

In a research to develop food and preventive treatment, the effect of Black Ginger Extract on spontaneously obese type II diabetic mice was examined. As shown in Fig. 14, Fig. 15 & Fig. 16 weight gain, visceral fat accumulation and blood sugar level were suppressed respectively in mice consuming Black Ginger Extract (1% and 3%) containing feed for 8 weeks. Meanwhile, no effect on above mentioned parameters were observed in normal mice (i.e. non-obese mice). It is suggestive that Black Ginger Extract is valuable as alternative preventive treatment for metabolic syndrome.

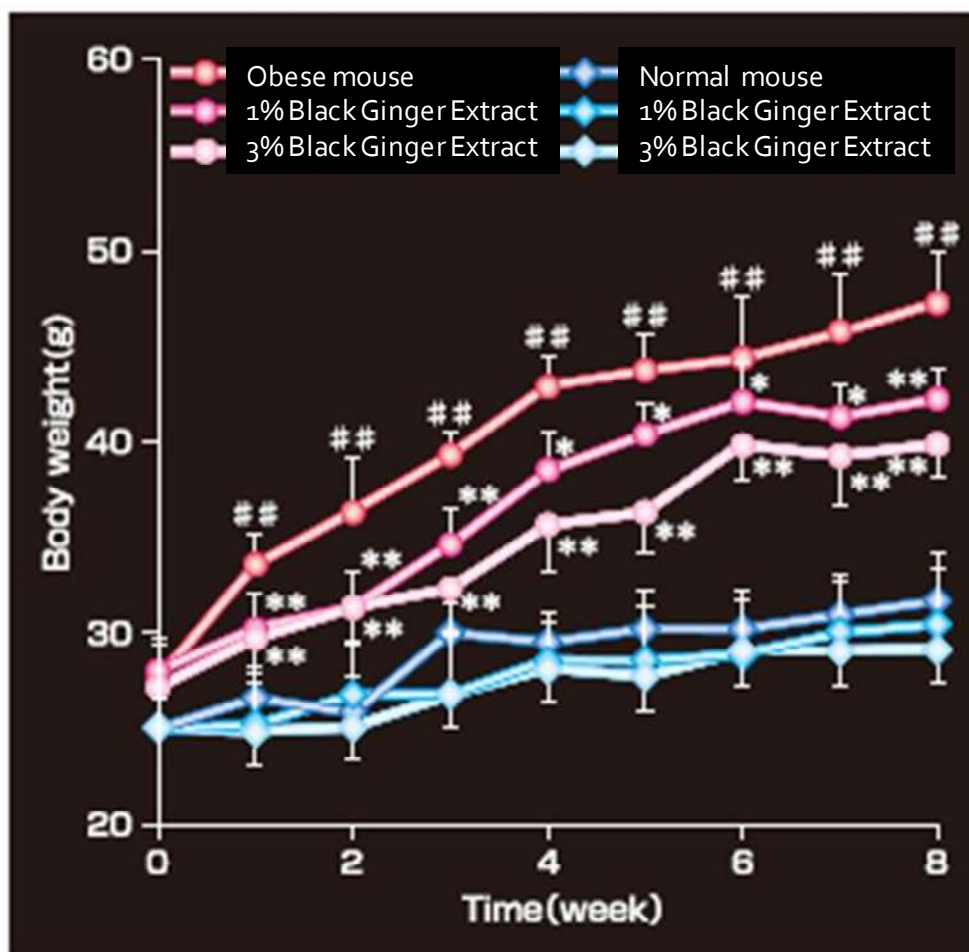
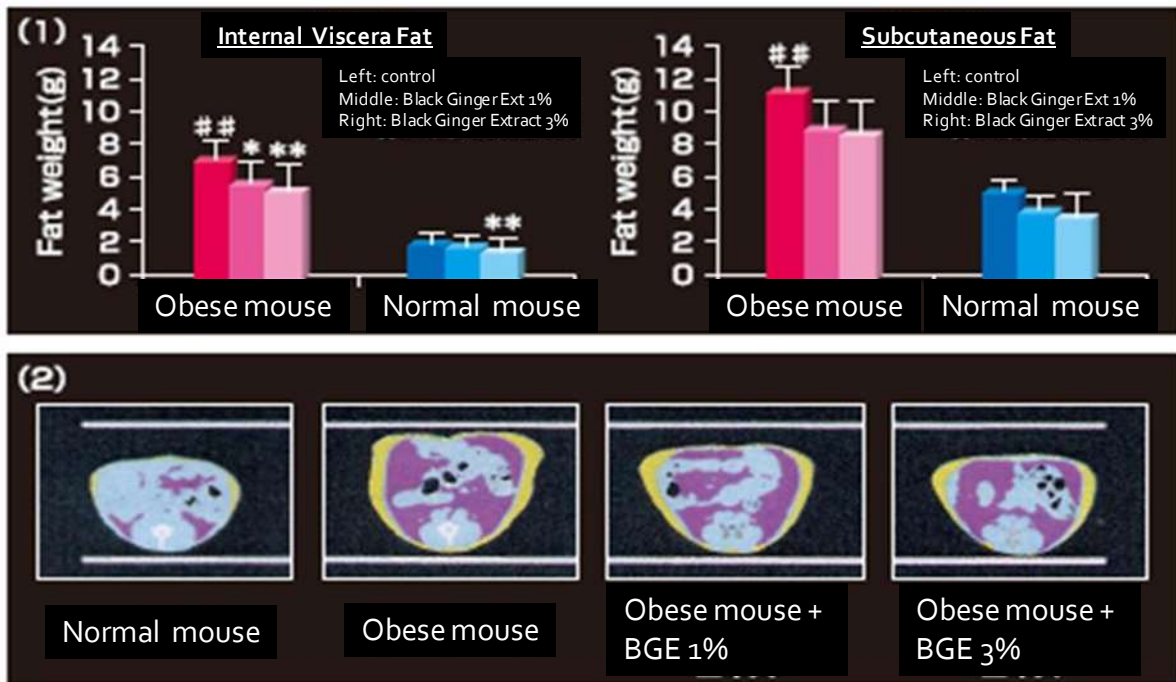


Fig. 14 Effect of Black Ginger Extract on mice weight changes over time



1. Effect on visceral fat and subcutaneous fat
2. CT Scan: purple: visceral fat, yellow: subcutaneous fat

Fig. 15 Effect of Black Ginger Extract on the accumulation of adipose tissue in spontaneously obese type II diabetic mice and normal mice.

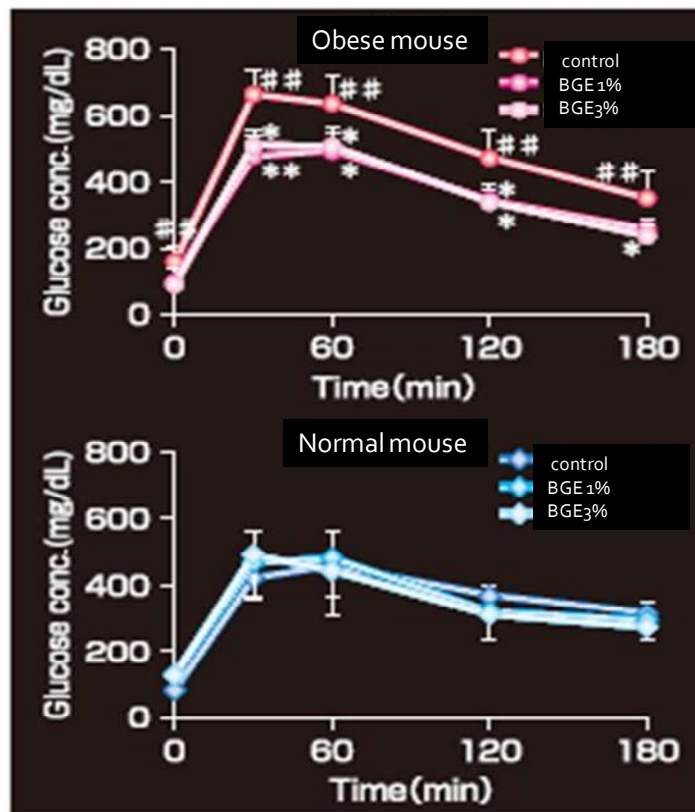
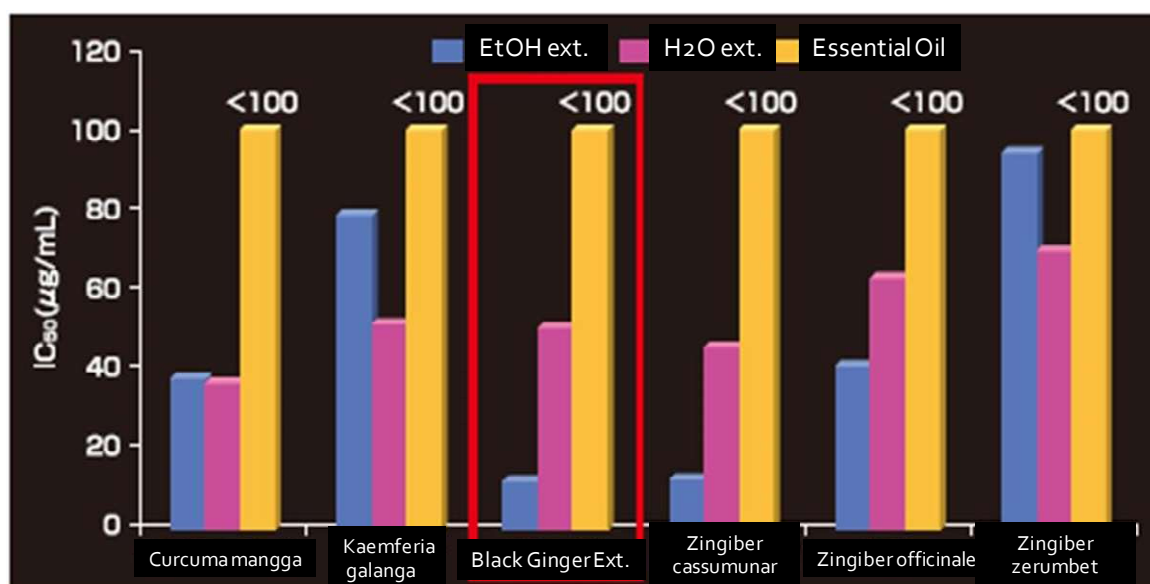


Fig. 16 Effect of Black Ginger Extract on Blood Sugar Level

Reference: T. Akase *et al. J Nat. Med.* **65**:73-80 (2011)

(5) Anti-allergy Effect

Tewtrakul *et al.*, conducted an investigation examining and comparing the anti-allergic activity of selected Zingiberaceous plants (including Black Ginger Extract) using RBL-2H3 cell line. Upon mast cell degranulation, the enzyme β -hexosaminidase is released along with histamine, thus a biomarker for antigen induced degranulation in rat basophil. As shown in Fig. 17, the ethanolic extract of Black Ginger demonstrated the most potent anti-allergic effect on prevention of mast cells degranulation with an IC_{50} of about 10 $\mu\text{g/ml}$ among the Zingingeraceous plants.



Among 6 different plant extract from ginger family, Black Ginger Extract (EtOH ext.) demonstrated the most potent activity on the inhibition of mast cells degranulation.

Fig. 17 Inhibitory effect of Black Ginger Extract on mast cell degranulation using RBL-2H3 cell line

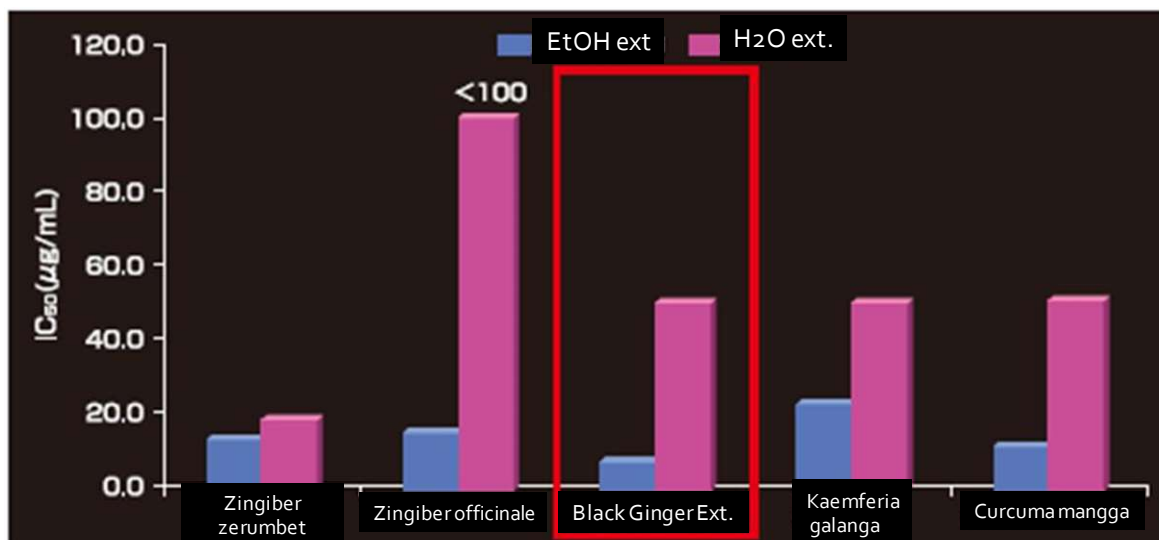
Reference:

S. Tewtrakul *et al., J Ethnopharmacol.*, **109**(3):535-8, (2007)

(6) Anti-inflammatory Effect

The anti-inflammatory effect of Black Ginger Extract and 5 other Zingiberaceous plants was examined using RAW264.7 macrophage cells where inhibitory activity on E.coli derived

lipopolysaccharides (LPS) –induced NO release in RAW264.7 cells was investigated. Results showed that ethanolic extract of Black Ginger exhibited potent inhibition on NO production in RAW264.7 macrophage cells, thus potent anti-inflammatory effect (Fig. 18).



Among 5 different plant extract from ginger family, Black Ginger Extract (EtOH ext.) is the most potent inhibitor of NO production in RAW 264.7 cells, hence, Black Ginger Extract is anti-inflammatory.

Fig. 18 Inhibitory effect of Black Ginger Extract on NO production in RAW264.7 macrophage cells

Reference:

S. Tewtrakul *et al.*, *J Ethnopharmacol.*, **120**:81-84, (2008)

(7) Improve Brain Function

As described above, phosphodiesterases (PDEs) degrade phosphodiester bond in the second messenger molecule cAMP and cGMP, therefore important regulators of signal transduction mediated by these second messenger molecules. PDE2 is highly expressed in the brain and adrenal glands (SH Francis *et al.*, *Prog Nucleic Acid RES Mol Biol.* **65**:1-52, 2001). The expression of mRNA of PDE has been identified in the hippocampus and cerebral cortex of the brain of rodents (WC Van Staveren *et al.*, *J Comp Neurol.*, 467:566-580, 2003). In a study conducted by Boess *et al.*, reported that administration of PDE2 inhibitor BAY 60-7550 in rats improved memory function by potentiating long-term increase in nerve cells rats (FG Boess *et al.*, *Neuropharmacology*, **47**:1081-1092, 2004).

The effect of Black Ginger Extract on PDE2 was examined. As shown in Fig. 19, Black Ginger Extract exhibited inhibitory effect on PDE2 at concentration of 1µg/mL and 10µg/mL.

Similarly, the standardized compound of Black Ginger Extract, 5,7-dimethoxyflavone inhibited PDE2 activity at concentration of 3 μ M and 30 μ M, hence the PDE2 inhibitory effect of Black Ginger Extract is contributed by 5,7-dimethoxyflavone. However, upon comparison with Piper Longum Extract, a health food ingredient with blood circulation enhancing effect, did not show inhibitory effect on PDE2 at low concentration of 10 μ g/mL but only at higher concentration of 100 μ g/mL (Fig. 19). As a result, Black Ginger Extract demonstrated 100 times more potent effect on the inhibition of PDE2 compared with Piper Longum Extract.

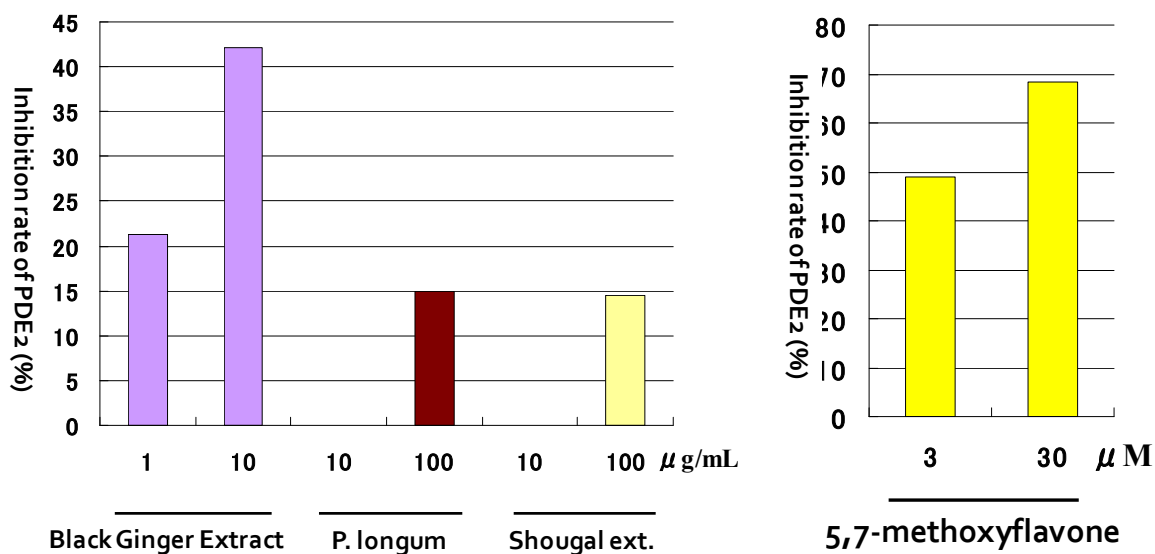


Fig. 19 Inhibitory Effect of Black Ginger Extract and 5,7-dimethoxyflavone on PDE2

(8) Antioxidant Effect

In a biological system, reactive oxygen species (e.g. superoxide and hydroxyl radicals) are generated in response to oxidative stress contributing to the development of various degenerative diseases e.g. cancer, inflammation, and ageing.

The antioxidant effect of Black Ginger Extract on Superoxide Dismutase (SOD) model and DPPH radical scavenging model was examined. As shown in Fig. 20, Black Ginger Extract demonstrated dose-dependent antioxidant effect on both SOD and DPPH radical scavenging models.

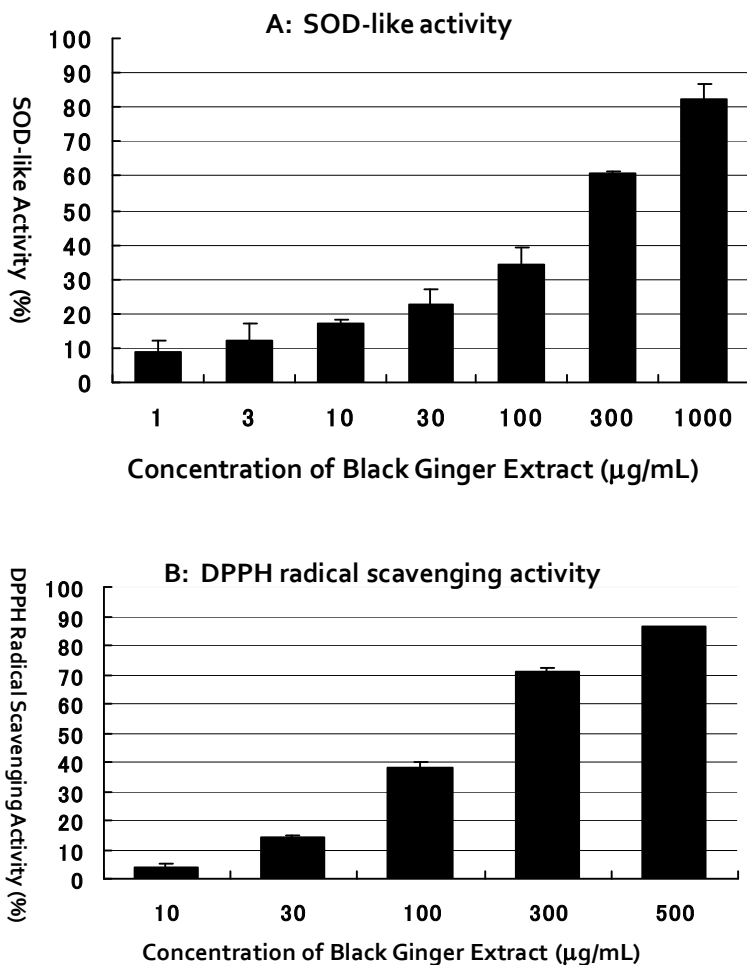


Fig. 20 Antioxidant effects of Black Ginger Extract

(9) Enhancement of female hormone (estradiol) in female mice

Black ginger extract was evaluated for positive effects on a menopausal disorder or anti-aging in a skin. Firstly, it was measured serum estradiol, one of the female hormones, using female mice. As you can see Fig. 21, black ginger extract significantly increased the serum estradiol compared with control in a dose dependent manner.

A lot of research regarding estradiol indicated that it related to various symptoms and disease such as menopausal disorder, anti-aging in a skin, arteriosclerosis, hyperlipidemia, osteoporosis, sterility and so on... therefore, black ginger extract was expected to improve or prevent these symptoms and disease.

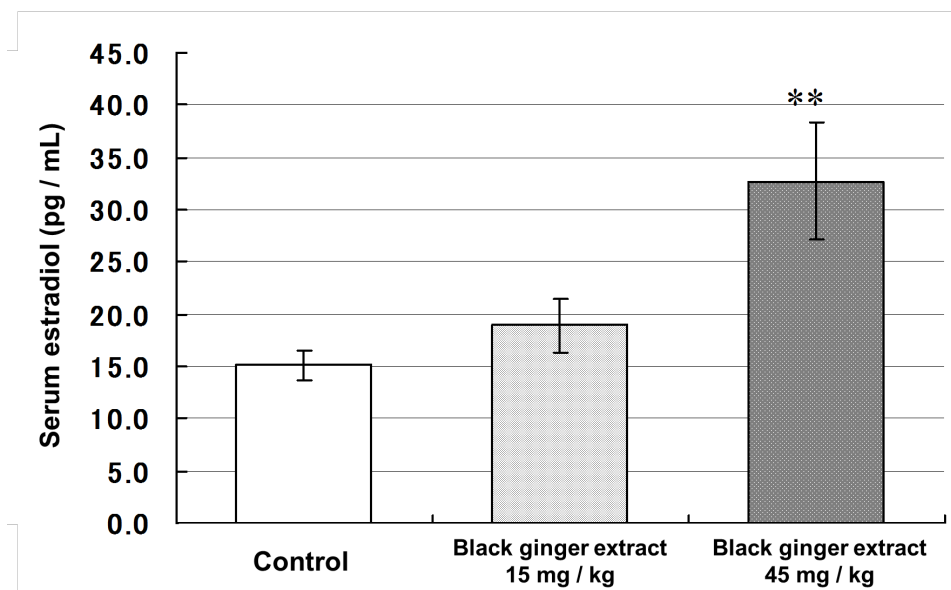


Figure 21. Effect of black ginger extract on increase in the serum estradiol in the female mice (n = 6, mean±S.E).

Experimental methods:

18 female mice, aged 6 or more months old, were divided into three groups with 6 mice in each group, control, black ginger extract (15 mg/kg) and black ginger extract (45 mg/kg). The mice were orally administered black ginger extracts or vehicle for 2 weeks, and then the serum, spleen and thymic were obtained from these mice after the final ingestion. The serum estradiol was measured using Estradiol ELISA kit (Fig. 21). In addition, it was measured the volumes of spleen and thymic in the mice (Fig. 22).

(10) Improvement of immunity

The positive effect of black ginger extract on immunity was evaluated using the above mice. The mice ingested black ginger extract was performed to measure the volumes of spleen and thymic (the organ was related to immunity such as differentiation of T-cells) in the mice.

As a result, black ginger extract (ingested at 45 mg/kg) increased the volume of these organs compared with control (Fig. 22). Therefore, black ginger extract was suggested to improve an immunity.

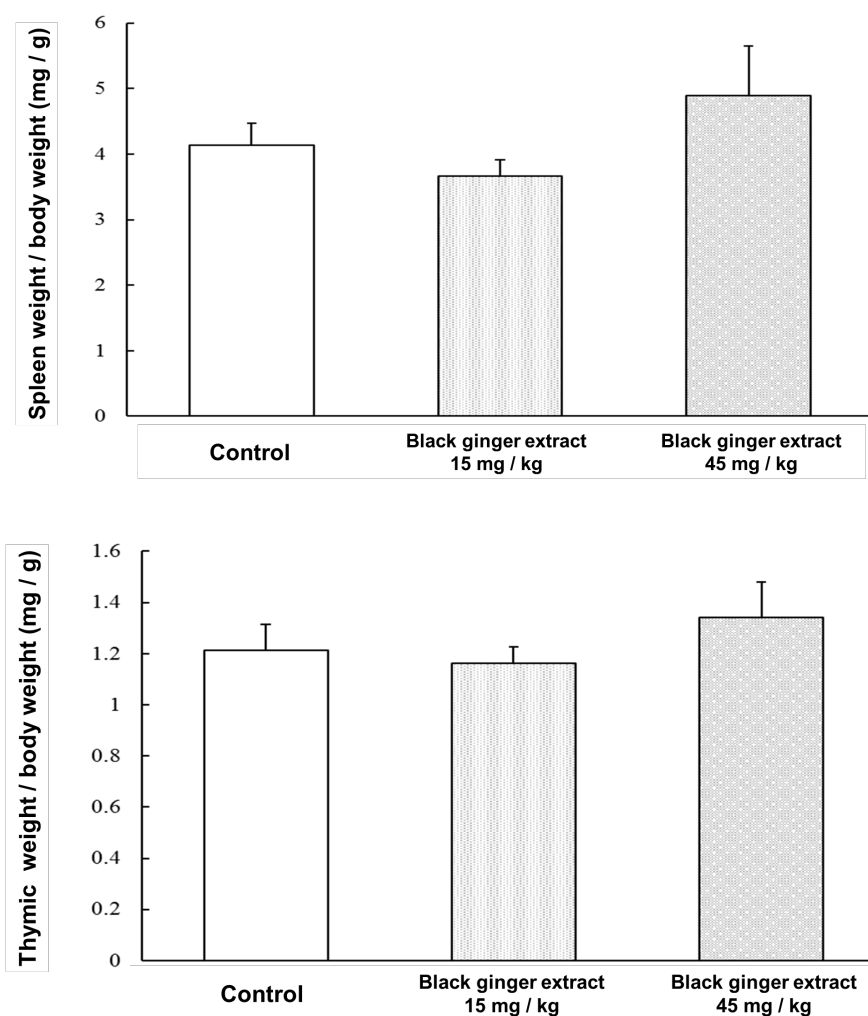


Figure 22. Effect of black ginger extract on increase in the volumes of spleen and thymic in the mice ($n = 6$, mea $n \pm S.E$).

4. Stability of Black Ginger Extract

(1) Heat Stability

The heat stability of Black Ginger Extract-P was examined by heating at 100°C and 120°C continuously for 1 hour. As shown in Fig. 23, content of 5,7-dimethoxyflavone, the principal component of Black Ginger Extract-P, and content of total flavonoids were not reduced after heating for 1 hour. Therefore, Black Ginger Extract-P is highly stable upon heating at normal food processing temperature.

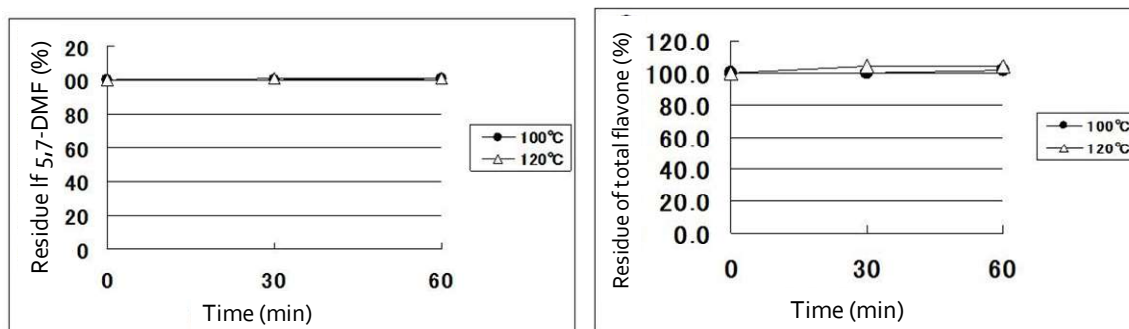


Fig. 23 The heat stability of Black Ginger Extract-P

5. Recommended Dosage

In accordance to the result of human clinical trials, the recommended dosage of Black Ginger Extract-P is 50-150mg/day.

6. Nutritional Profile

Analyzed Item	Black Ginger Extract-P	Black Ginger Extract-WSP	Analysis Method	Remarks
Energy	407 kcal/100g	410kcal/100g	Modified Atwater method	1
Protein	3.6g/100g	0.6g/100g	Kjeldahl method	2
Fatty Acid	5.3g/100g	0.9g/100g	Acid degradation	
Sugar	85.5g/100g	97.6g/100g	Calculation: 100 – (water + protein + fat + ash)	3
Ash	1.0g/100g	0.2g/100g	Direct incineration	
Water	3.3g/100g	0.6g/100g	Heat drying at atmospheric pressure	
Fiber	1.0g/100g	0.2g/100g	Prosby method	
Sodium	66.3mg/100g	11.1mg/100g	Atomic absorption spectrophotometry	
Sodium chloride equiv.	0.17g/100g	0.03g/100g	Sodium equiv. value	

Remarks:

1. Energy conversion: protein 4, fat 9, sugar 4, fiber 2
2. Protein conversion factor: 6.25
3. Calculation: 100 – (water + protein + fat + ash)
4. Nutritional Value of Black Ginger Extract-WSP is calculated from the Nutritional value of Black Ginger Extract-P

Test Trustee: Food Analysis Technology Centre SUNATEC

Date: May 15, 2012

Report No.: 120501169-001-01

7. Safety Profile

(1) Residual Agricultural Chemicals

Black Ginger Extract (without binder) was screened and analyzed for residual agricultural chemicals (535 items) stipulated under the Food Sanitation Act and Pesticides Control Act, presence of the test items was lower than the allowed limits.

Test Trustee: Masis Co., Ltd.; Center for Food Safety Evaluation and Analysis

Date: May 17, 2012

Report No.: 54521

(2) Acute Toxicity (LD₅₀)

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where Black Ginger Extract (without binder) 2000mg/kg was orally given to mice (male & female ICR, 5 weeks old, weight 20-25g) for 14 days. The mice were housed at $23 \pm 2^\circ\text{C}$ and at $50 \pm 10\%$ humidity with free access to feed and drinking water for 14 days. No abnormal change was found in their weight as compared to the control group. No abnormalities were found in their organs upon autopsy after the test either. LD₅₀ of Black Ginger Extract is deduced to be 2,000 mg/kg.

(3) Mutagenicity (Ames Test)

Ames test was conducted to evaluate the mutagenicity of Black Ginger Extract (without binder) using *Salmonella typhimurium* strain TA98 and TA100. There was no increase in the number of colonies ($19.5 \sim 5000 \mu\text{g} / \text{plate}$) in both direct method and metabolism activation method. Black Ginger Extract was considered as non-mutagenic.

(4) The safety in case of excessive consumption (5 times higher dosage)

We evaluated safety of excessive consumption of black ginger extract-P (500 mg / day). The study evaluated at double blind parallel test in healthy male and female aged from 23 to 72 years old. Questionnaire and measurements of blood pressure, pulse count, blood parameter, urinary parameter and subjective symptoms were performed. As a result of 4-week treatments any abnormal changes were not observed in all parameters. Furthermore there were no dropouts and subjects complaining health problem.

8. Applications

	Applications Claims Examples	Claims	Examples
Food Nutritional Supplement	Food Nutritional Supplement	<ol style="list-style-type: none"> 1. Improve blood circulation 2. Relief edema 3. Improve vitality 4. Anti-obesity 	Beverages Hard & soft capsules, tablets Candies, chewing gums, chocolates, wafers, jellies Ham, sausage, etc.
Beauty Food Eye health	Beauty Food Eye health	<ol style="list-style-type: none"> 5. Anti-inflammatory 6. Beauty food 7. Cosmetics 	Lotions, toner, serum, rinse, treatment care, pack, body gel etc.

9. Packing

Black Ginger Extract-P (powder, food grade)

Black Ginger Extract-WSP (water soluble powder, food grade)

Black Ginger Extract-PC (powder, cosmetics grade)

Black Ginger Extract-WSPC (water soluble powder, cosmetics grade)

1kg, 5kg interior packing: Aluminium bag

Exterior packing: Cardboard box

Black Ginger Extract-LC (liquid, cosmetics grade)

1kg, 5kg Interior packing: Tin can

Exterior packing: Cardboard box

10. Storage

Store in a cool, dry and dark place. Avoid heat and places with high humidity.

11. Expression of Black Ginger Extract

Food grade:

Black Ginger Extract-P

Expression: Black Ginger Extract and modified starch

Black Ginger Extract-WSP

Expression: Black Ginger Extract and cyclodextrin

It is suggested to reconfirm with the Regional Agricultural Administration Office for public health and food labeling.

Cosmetic grade:

Black Ginger Extract-PC

INCI name: Kaempferia Parviflora Rhizome Extract (and) Starch Sodium Octenyl Succinate (application in progress)

Expression: Black Ginger Extract (application in progress), Starch Sodium Octenyl Succinate

Black Ginger Extract-WSPC

INCI name: Kaempferia Parviflora Rhizome Extract (and) Maltosyl Cyclodextrin (and) Cyclodextrin (and) Maltose (application in progress)

Expression: Black Ginger Extract (application in progress), Maltosyl Cyclodextrin and Cyclodextrin and Maltose

Black Ginger Extract-LC

INCI name: Kaempferia Parviflora Rhizome Extract (and) Propanediol (application in progress)

Expression: Black Ginger Extract (application in progress), Propanediol

PRODUCT STANDARD

PRODUCT NAME : **BLACK GINGER EXTRACT-P** (FOOD)

This product is extracted with aqueous ethanol from the rhizome of *Kaempferia parviflora* (*Zingiberaceae*). It contains a minimum of 2.5 % 5,7-dimethoxyflavone and 10.0 % total flavonoids.

<u>Appearance</u>	Dark purple to dark brown powder with light unique aroma									
<u>5,7-Dimethoxyflavone</u>	Min. 2.5 %	(HPLC)								
<u>Total Flavonoids</u>	Min. 10.0 %	(Spectrophotometry)								
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 °C, 2 hr)								
<u>Purity Test</u>										
<u>(1) Heavy Metals (as Pb)</u>	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)								
<u>(2) Arsenic (as As₂O₃)</u>	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)								
<u>Standard Plate Counts</u>	Max. 1×10 ³ cfu/g	(Analysis for Hygienic Chemists)								
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)								
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
<u>Composition</u>	<table border="0" style="width: 100%;"> <thead> <tr> <th style="text-align: left;"><u>Ingredient</u></th> <th style="text-align: right;"><u>Content</u></th> </tr> </thead> <tbody> <tr> <td>Modified Starch</td> <td style="text-align: right;">70 %</td> </tr> <tr> <td><u>Black Ginger Extra</u></td> <td style="text-align: right;">30 %</td> </tr> <tr> <td>Total</td> <td style="text-align: right;">100 %</td> </tr> </tbody> </table>		<u>Ingredient</u>	<u>Content</u>	Modified Starch	70 %	<u>Black Ginger Extra</u>	30 %	Total	100 %
<u>Ingredient</u>	<u>Content</u>									
Modified Starch	70 %									
<u>Black Ginger Extra</u>	30 %									
Total	100 %									

PRODUCT STANDARD

PRODUCT NAME : **BLACK GINGER EXTRACT-WSP** (FOOD)

This product is extracted with aqueous ethanol from the rhizome of *Kaempferia parviflora* (*Zingiberaceae*). It contains a minimum of 0.25 % 5,7-dimethoxyflavone and 1.00 % total flavonoids. This product is water soluble.

<u>Appearance</u>	Greyish brown to greyish purple powder with light unique aroma									
<u>5,7-Dimethoxyflavone</u>	Min. 0.25 %	(HPLC)								
<u>Total Flavonoids</u>	Min. 1.00 %	(Spectrophotometry)								
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 °C, 2 hr)								
<u>Purity Test</u>										
<u>(1) Heavy Metals (as Pb)</u>	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)								
<u>(2) Arsenic (as As₂O₃)</u>	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)								
<u>Standard Plate Counts</u>	Max. 1×10 ³ cfu/g	(Analysis for Hygienic Chemists)								
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)								
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
<u>Composition</u>	<table border="0" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left; border-bottom: 1px solid black;"><u>Ingredient</u></th> <th style="text-align: right; border-bottom: 1px solid black;"><u>Content</u></th> </tr> </thead> <tbody> <tr> <td>Cyclodextrin</td> <td style="text-align: right;">95 %</td> </tr> <tr> <td><u>Black Ginger Extract</u></td> <td style="text-align: right;"><u>5 %</u></td> </tr> <tr> <td>Total</td> <td style="text-align: right;">100 %</td> </tr> </tbody> </table>		<u>Ingredient</u>	<u>Content</u>	Cyclodextrin	95 %	<u>Black Ginger Extract</u>	<u>5 %</u>	Total	100 %
<u>Ingredient</u>	<u>Content</u>									
Cyclodextrin	95 %									
<u>Black Ginger Extract</u>	<u>5 %</u>									
Total	100 %									

PRODUCT STANDARD

PRODUCT NAME : **BLACK GINGER EXTRACT-PC** (COSMETIC)

This product is extracted with aqueous ethanol from the rhizome of *Kaempferia parviflora* (*Zingiberaceae*). It contains a minimum of 2.5 % 5,7-dimethoxyflavone and 10.0 % total flavonoids.

<u>Appearance</u>	Purple powder with light unique aroma.	
<u>5,7-Dimethoxyflavone</u>	Min. 2.5 %	(HPLC)
<u>Total Flavonoids</u>	Min. 10.0 %	(Spectrophotometry)
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 °C、 2 hr)

Purity Test

(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As₂O₃)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)

Standard Plate Counts Max. 1×10² cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10² cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredient</u>	<u>Content</u>
Starch Sodium Octenyl Succinate	70 %
<u>Kaempferia Parviflora Rhizome Extract</u>	<u>30 %</u>
Total	100 %

PRODUCT STANDARD

PRODUCT NAME : **BLACK GINGER EXTRACT-WSPC** (COSMETIC)

This product is extracted with aqueous ethanol from the rhizome of *Kaempferia parviflora* (*Zingiberaceae*). It contains a minimum of 0.25 % 5,7-dimethoxyflavone and 1.00 % total flavonoids. This product is water soluble.

<u>Appearance</u>	Light Purple powder with light unique aroma.	
<u>5,7-Dimethoxyflavone</u>	Min. 0.25 %	(HPLC)
<u>Total Flavonoids</u>	Min. 1.00 %	(Spectrophotometry)
<u>Loss on Drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 °C、 2 hr)
<u>Purity Test</u>		
(1) Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2) Arsenic (as As₂O₃)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
<u>Standard Plate Counts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	<u>Ingredient</u>	<u>Content</u>
	Maltosyl Cyclodextrin	} 95 %
	Cyclodextrin	
	Dimaltosyl Cyclodextrin	
	Maltose	
	<u>Kaempferia Parviflora Rhizome Extract</u>	<u>5 %</u>
	Total	100 %

PRODUCT STANDARD

PRODUCT NAME : **BLACK GINGER EXTRACT-LC** (COSMETIC)

This product is extracted from the rhizome of *Kaempferia parviflora* (*Zingiberaceae*), with aqueous ethanol and is dissolved in propanediol. It guarantees minimum 0.1% 5,7-dimethoxyflavone and 0.4% total flavonoids.

<u>Appearance</u>	Reddish brown to purple liquid with slightly characteristic odor..	
<u>5,7-Dimethoxyflavone</u>	Min. 0.1 %	(HPLC)
<u>Total Flavonoids</u>	Min. 0.4 %	(Spectrophotometry)
<u>Purity Test</u>		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As₂O₃)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
<u>Standard Plate Counts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	<u>Ingredient</u>	<u>Content</u>
	Propanediol	90 %
	Water	9 %
	<u>Kaempferia Parviflora Rhizome Extract</u>	<u>1 %</u>
	Total	100 %

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

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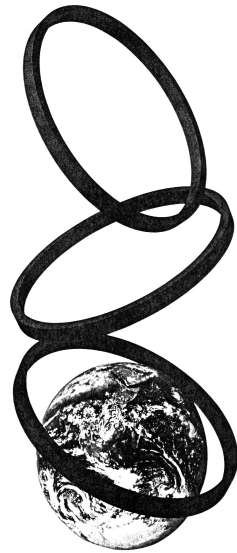
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