Oryza

ORYZA OIL & FAT CHEMICAL CO., LTD.

COFFEE BEAN EXTRACT

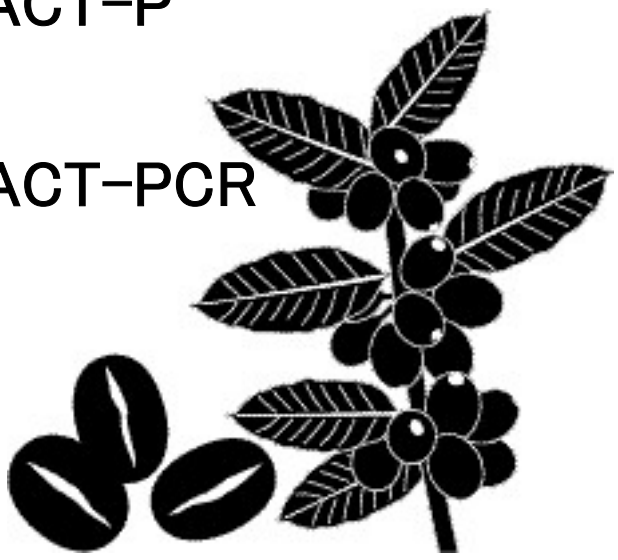
Health Ingredient For Prevention Against Obesity, Diabetes Mellitus & Maintenance of Healthy Weight

■COFFEE BEAN EXTRACT-P

(Powder, Food grade)

■COFFEE BEAN EXTRACT-PCR

(Powder, Cosmetic grade)



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1. Introduction

Overweight refers to increased body weight in relation to height, when compared to some standard of acceptable or desirable weight. **Obesity** is defined as an excessively high amount of body fat or adipose tissue in relation to lean body mass. Obesity and overweight emerged as chronic conditions and are contributing factors of many preventable illnesses, e.g., diabetes mellitus, coronary artery disease, high blood pressure etc.

A recent survey in Japan (2002) indicated that incidence of diabetes mellitus is increasing. While in the United States, obesity has risen at an epidemic rate during the past 20 years. Overweight & obesity and associated health problems created a significant economic impact on health care system in the world. Poor diet and/or lack of physical activities are common causes of obesity.

Therapeutic agents, food supplements are developed to treat and prevent obesity. Chitosan, one of the most popular food supplements for weight loss claimed to reduce fat absorption, citrus (*Citrus aurantium*) fruits assist in breaking down fat while capsaicin improve fat metabolism. These ingredients are widely used in health food supplements and as functional food for combating obesity and associated diseases. On the other hand, herbal supplements have been developed for the prevention of Diabetes Mellitus, e.g., guava leaves (*Psidium guajava*) and banaba leaves (*Lagerstroemia speciosa*). Some of the herbal ingredients have been approved in Japan for application in food preparations for maintenance of general health and well being.

Oryza Oil & Fat Chemicals Co., Ltd. has identified the beneficial effect of coffee bean extract in preventing obesity and diabetes mellitus. **Chlorogenic acid** is found in high concentration in coffee beans, has recently been identified as a selective inhibitor for the production of glucose in liver[1)]. It was found that raw coffee beans consist of higher concentration of chlorogenic acid as compared to roasted coffee beans[2)]. Meanwhile, **caffeine**, the main component in coffee enhances physical endurance & capabilities, hence promotes energy utilization and lipolysis.

Oryza Oil & Fat Chemical Co., Ltd. has successfully commercialized the production of “Coffee Bean Extract” which is highly water soluble and suitable to be incorporated into various functional food preparations.

1) Schwab D. *et al*., Hepatic uptake of synthetic chlorogenic acid derivatives by the organic anion transport proteins. *J. Pharmacol. Exp. Ther.* **296**, 91-8 (2001).
2) Moon J. K. *et al*., Role of roasting conditions in the level of chlorogenic acid content in coffee beans: correlation with coffee acidity. *J. Agric. Food Chem.* **57**, 5365-9 (2009).

Raw coffee beans

Fruits of coffee (coffee cherry)

2. The Effects of coffee

a. Coffee as medicinal compound – historical approach

History of coffee was dated back to the Islamic world in 11th century where coffee was used as a medicinal compound for gastric disorders by Rhases, an Islamic physician. In the 16th century, coffee was officially introduced to Europe as a beverage with stimulating effects on the central nervous system. In 19th century, coffee has become a potential substitute for alcohol due to its central stimulating effects. Today, caffeine is listed in Pharmacology textbooks as CNS stimulant and diuretic. In recent years, coffee generated enormous popularity and has been regarded as health drink*.

* Fukushima Y. *et al*., Coffee and green tea as a large source of antioxidant polyphenols in the Japanese population. *J. Agric. Food Chem.*, **57**, 1253-9 (2009).

b. Antioxidative effects

Epidemiological study conducted in Italy indicated that coffee has the greatest Total Antioxidant Capacity on comparison with 34 beverages. This is suggestive of its psychological/oxidative stress alleviating effect.

Pellegrini N., et al., Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr*. **133**, 2812-9 (2003).

c. Prevention against liver cirrhosis

Acetaldehyde has been documented to cause adverse effects in the liver. This includes depletion of liver glutathione, vitamins and minerals. Caffeine, a member of methylxanthine alkaloids, is capable of degrading and excreting acetaldehyde from alcohol. Study confirmed an inverse relation between coffee consumption and liver cirrhosis[1)] and viral hepatitis[2)].

1) *Tverdal A., et al.,* Coffee intake and mortality from liver cirrhosis. *Ann. Epidemiol*. **13**, 419-23 (2003).
2) Inoue M., *et al.*, Effect of coffee and green tea consumption on the risk of liver cancer: cohort analysis by hepatitis virus infection status. *Cancer Epidemiol. Biomarkers Prev.* **18**, 1746-53 (2009).

d. Prevention against atherosclerosis

Low levels of high-density lipoprotein (HDL) often associated with increase risk of atherosclerosis. A study on the effect of coffee in gastric potential difference showed that total cholesterol & high-density lipoprotein (HDL) cholesterol are significantly higher in subjects consuming coffee. Coffee is thus potentially useful in preventing atherosclerosis.

Ehlirch A., et al., Effect of processed and non-processed coffee samples on gastric potential difference. Study with healthy male H. pylori-positive and *H.pylori*-negative volunteers. *Arzneimittelforschung* **49,** 626-30 (1999).

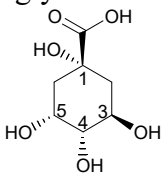
e. Prevention against rectal diseases

Study conducted at Division of Epidemiology, Aichi Cancer Center Research Institute, Nagoya, Japan showed that the risk of rectal cancer reduces with daily intake of 3 cups or more of coffee. This is believed to be associated with anti-tumor effect of chlorogenic acid. The results suggested the potential for prevention against site-specific digestive tract cancer by consumption of coffee.

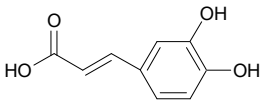
Inoue M., et al., Tea and coffee consumption and the risk of digestive tract cancers: data from a comparative case referent study in Japan. *Cancer Causes Control* **9**, 209-16 (1998). *Jiang Y., et al.,* Induction of cytotoxicity by chlorogenic acid in human oral tumor cell lines. *Phytomedicine* **7,** 483-91 (2000). Oba S., *et al.*, Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes in Japanese men and women. *Br. J. Nutr.* **103**, 453-9 (2010).

3. Functional Components of Raw Coffee Beans

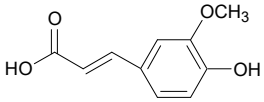
Raw coffee beans rich in **chlorogenic acid** and its related compound such as *quinic acid*, *p*-coumaric acid, and caffeic acid. Coffee is rapidly absorbed and reaches peak plasma concentration within 1 hour.[1] About 1/3 of chlorogenic acid absorbed orally and usually exist in the form of sulfates of caffeic acid or its glucuronide conjugates.[1,2] Studies indicated that chlorogenic acid inhibits the action of glucose-6-phosphatase, a rate-limiting enzyme involved in gluconeogenesis in the liver[3] & promotes insulin secretion.[4] Gluconeogenesis is usually prevented when glucose intake is restricted and this leads to muscle wastage. Thus, rebound effect of weight gain is greater when normal diet resumes. Clinical study reported that daily consumption of 90mg chlorogenic acid assist in a mean inhibition of 15%-20% in postprandial hyperglycemia.[5]



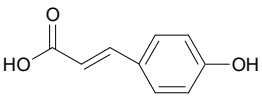
quinic acid



caffeic acid

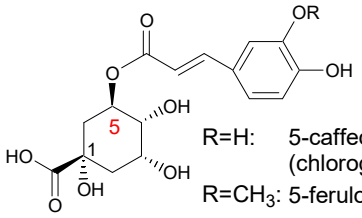


ferulic acid

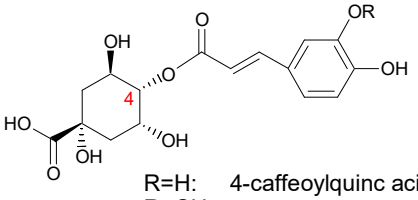


p-coumaric acid

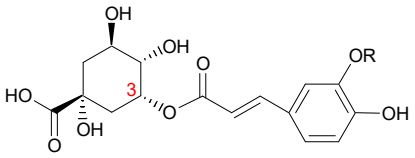
Chlorogenic acid related compounds



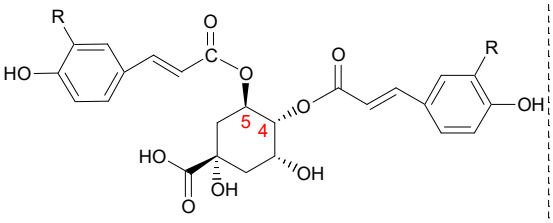
R=H: 5-caffeoylquinc acid (chlorogenic acid)
R=CH_3: 5-feruloylquinc acid



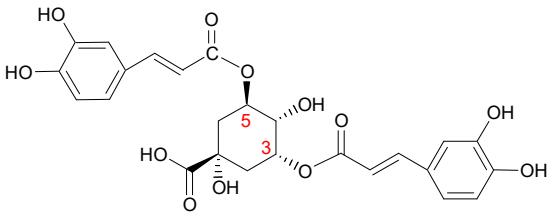
R=H: 4-caffeoylquinc acid
R=CH_3: 4-feruloylquinc acid



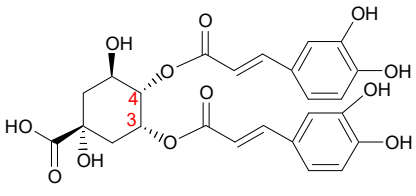
R=H: 3-monocaffeoylquinc acid (neochlorogenic acid)
R=CH_3: 3-feruloylquinc acid



R=OH: 4,5-dicaffeoylquinc acid
R=H 4,5-coumaroylquinc acid



R=OH: 3,5-dicaffeoylquinc acid
R=H 3,5-coumaroylquinc acid



R=OH: 3,4-dicaffeoylquinc acid
R=H 3,4-coumaroylquinc acid

Fig. 1. Functional components of raw coffee beans

In addition, caffeine, an ergogenic acid in coffee, has been shown to increase endurance, particularly in prolonged exercise duration lasting 30-120 mins,[6-8] mobilizes fatty acids from adipose tissue, spare carbohydrate stores and promotes endurance performance.[9,10] Reviews showed the effect of caffeine, carnitine and choline supplementation decreases body fat & serum leptin concentration.[11] Coffee as the most commonly available source of caffeine, potentially beneficial in assisting weight loss and weight maintenance.

The effects of caffeine on glucose metabolism and insulin sensitivity have been controversial. Recent findings shown that coffee consumption was associated with a substantially lower risk of Type II Diabetes Mellitus.[12]

References

1） Naldini M., *et al*. Absorption of phenolic acids in humans after coffee consumption. *J. Aglic. Food Chem.* **50**, 5735-41 (2002).

2） Olthof M.R., *et al*. Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* **131**, 66-71 (2001).

3) Arion W.J., *et al*. Chlorogenic acid and hydroxynitrobenzaldehyde:new inhibitors of hepatic glucose 6-phosphatase. *Arch. Biochem. Biophys.* **339**, 315-22 (1997).

4） Nomura H., *et al*. Acceleration of ferulic acid and related compounds on insulin secession . Research report of Wakayama industrial technology center 2001, 17-9 (2001).

5） Adidoff M.T., *et al*. Special clinical report for Russian Ministry of health. *Moscou, clinical report* 12 (1999).

6） Graham T.E., *et al*. Metabolic and exercise endurance effects of coffee and caffeine ingestion. *J. Appl. Physiol*. **85**, 883-9 (1998).

7） Graham, T.E., and L.L Sprilet. Performance and metabolic responses to high caffeine dose during prolong exercise. *J. Appl. Physiol*. **71**. 2292-2298 (1991).

8) Graham, T.E., *et al*. Caffeine and exercise, metabolism & performance. *Can. J. Appl. Physiol,* **19**, 111-138, (1994).

9) Greer F., *et al*. Caffeine ingestion decrease glucose disposal during a hyperinsulinemic-euglycemic clamp in sedentary humans. *Diabetes* **50**, 2349-54 (2001).

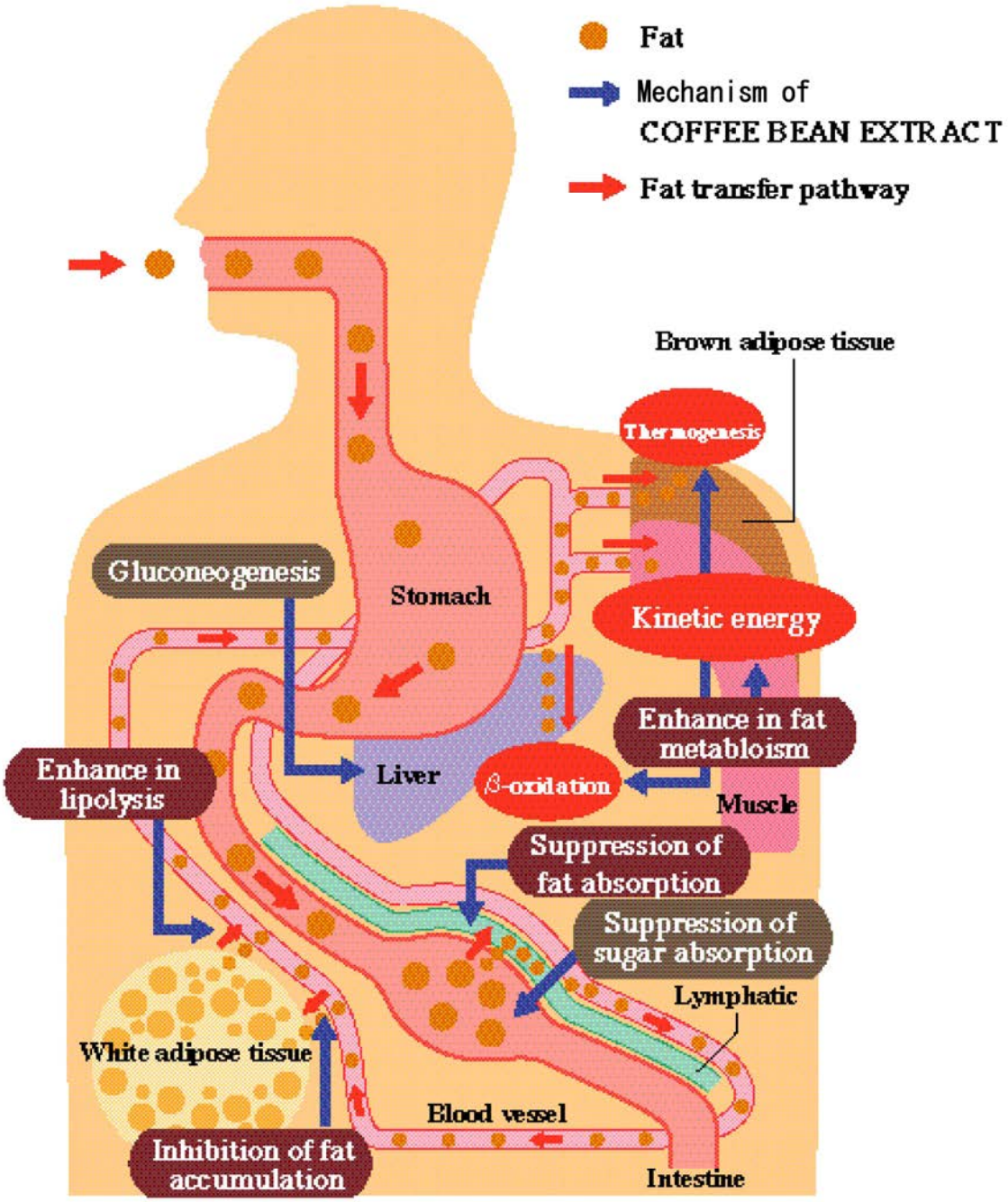
10） Ryu S., *et al*. Caffeine as a lipolytic food component increases endurance performance in rats and athletes. *J. Nutr. Sci. Vitaminol.* **47**, 139-46 (2001).

11） Hungu N., *et al*. Caffeine, carnitine and choline supplementation of rats decreases body fat and serum leptin concentration as dose exercise. *J. Nutr. Physiol.* **130**, 152-7 (2001).

12） Van Dam R.M., *et al*. Coffee consumption and risk of type 2 diabetes mellitus. *Lancet* **360**, 1477-8 (2002).

Health Promoting effects of COFFEE BEAN EXTRACT

Fat Metabolism &
Mechanism of Action of Coffee Bean Extract



4. Anti-obesity, anti-diabetic and anti-oxidative activities of COFFEE BEAN EXTRACT

(1) Prevention against fat absorption

a. Delays fat absorption (*in vivo*)

Coffee has been shown to inhibit GI motility (gastric emptying and peristalsis), Coffee Bean Extract is believed to delay fat absorption. The effect of Coffee Bean Extract on fat absorption was evaluated using single dose olive oil administration on mice. Fig. 2 illustrates that Coffee Bean Extract and caffeine significantly reduce serum triglyceride level while chlorogenic acid has no effect on serum triglyceride level. Coffee Bean Extract with its caffeine constituents suppresses fat absorption.

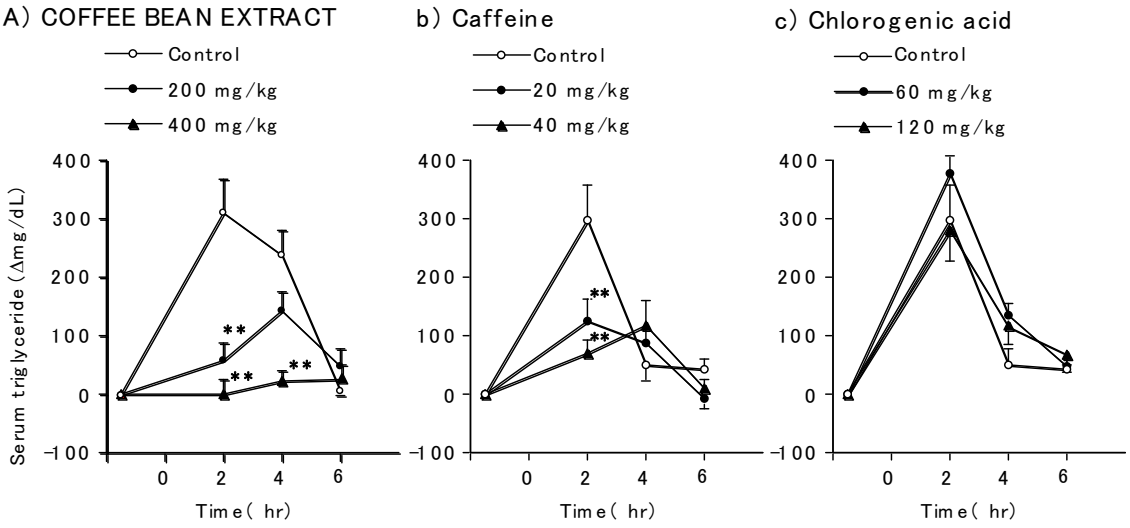


Fig. 2. Effect of COFFEE BEAN EXTRACT and its constituents on elevated serum triglyceride level in mice loaded with olive-oil.
[n=6, mean±S.E., **: $p<0.01$ (Dunnett's multiple range test)]

Materials and Methods:

Animals and Treatment. 6-wk old male ddY mice were fasted for 20 hours. 5%w/v gum Arabic suspension containing Coffee Bean Extract (10 ml/kg) were administered orally 30 minutes later followed by olive oil (5 ml/kg) 1 hour later.
Sample collections and Assays. Initial blood samples were collected prior to and at 2, 4 and 6 hour after administration of Arabic suspension and olive oil. Serum was separated and triglyceride concentration was determined using enzymatic method (Triglyceride E-Test Wako, Wako Pure Chemical Industries, Co., Ltd.)

b. Inhibits pancreatic lipase activity (*in vitro*)

The effect of Coffee Bean Extract and its functional components on pancreatic lipase activity was assessed and examined *in vitro*. As illustrated in Fig. 3, Coffee Bean Extract, chlorogenic acid and caffeine possess significant inhibitory effect on pancreatic lipase activity.

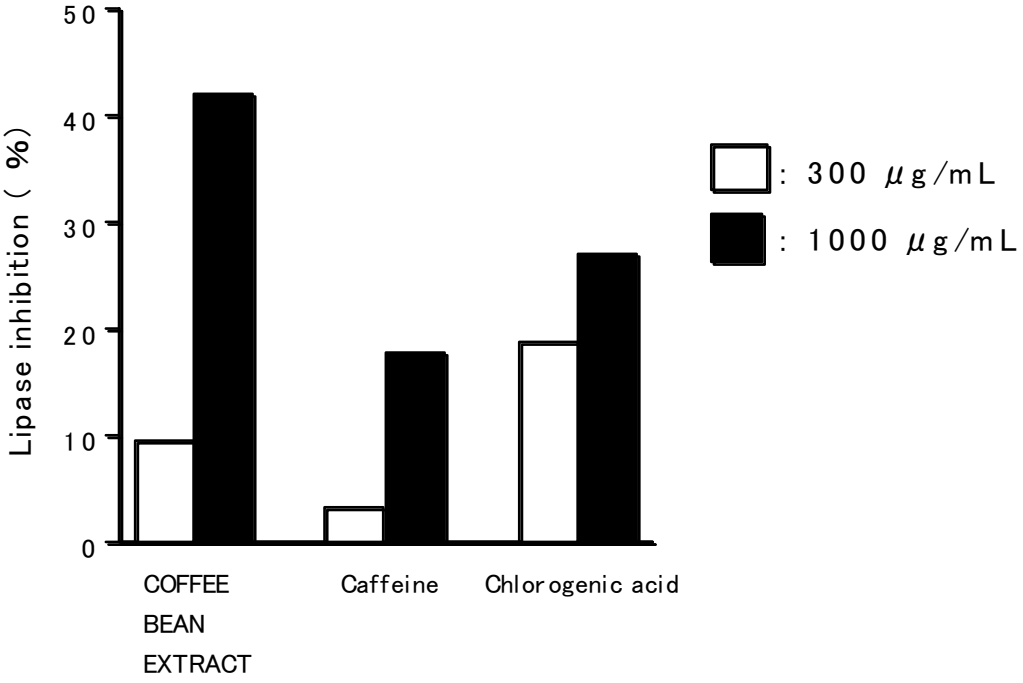


Fig.3. The effect of COFFEE BEAN EXTRACT and its constituents on pancreatic lipase activity.

Materials and methods:

Measurements and analysis. Porcine derived pancreatic lipase (by SIGMA: final concentration, 105.8 units/ml) was used. Inhibitory activity was measured using Lipase Kit-S (by Dainippon Pharmaceutical Co., Ltd.)

(2) Inhibition of fat accumulation
a. Suppresses differentiation of 3T3-L1 adipocytes (*in vitro*)

The inhibitory effect of Coffee Bean Extract on fat absorption has prompted further studies on accumulation of fat cell using mouse adipocyte strain (3T3-L1) culture system as illustrated in Fig. 4. Results confirmed that caffeine and chlorogenic acid inhibit fat cells accumulation in mouse adipocytes as illustrated in Fig. 5. Meanwhile, no toxicity observed at all concentration.

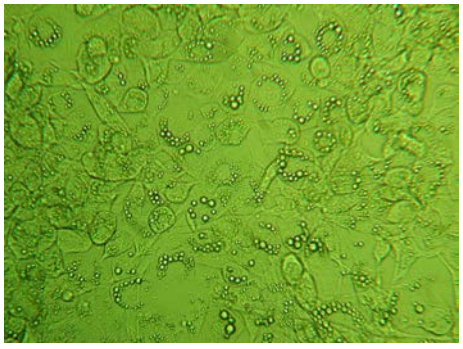


Fig. 4. Differentiated 3T3-L1

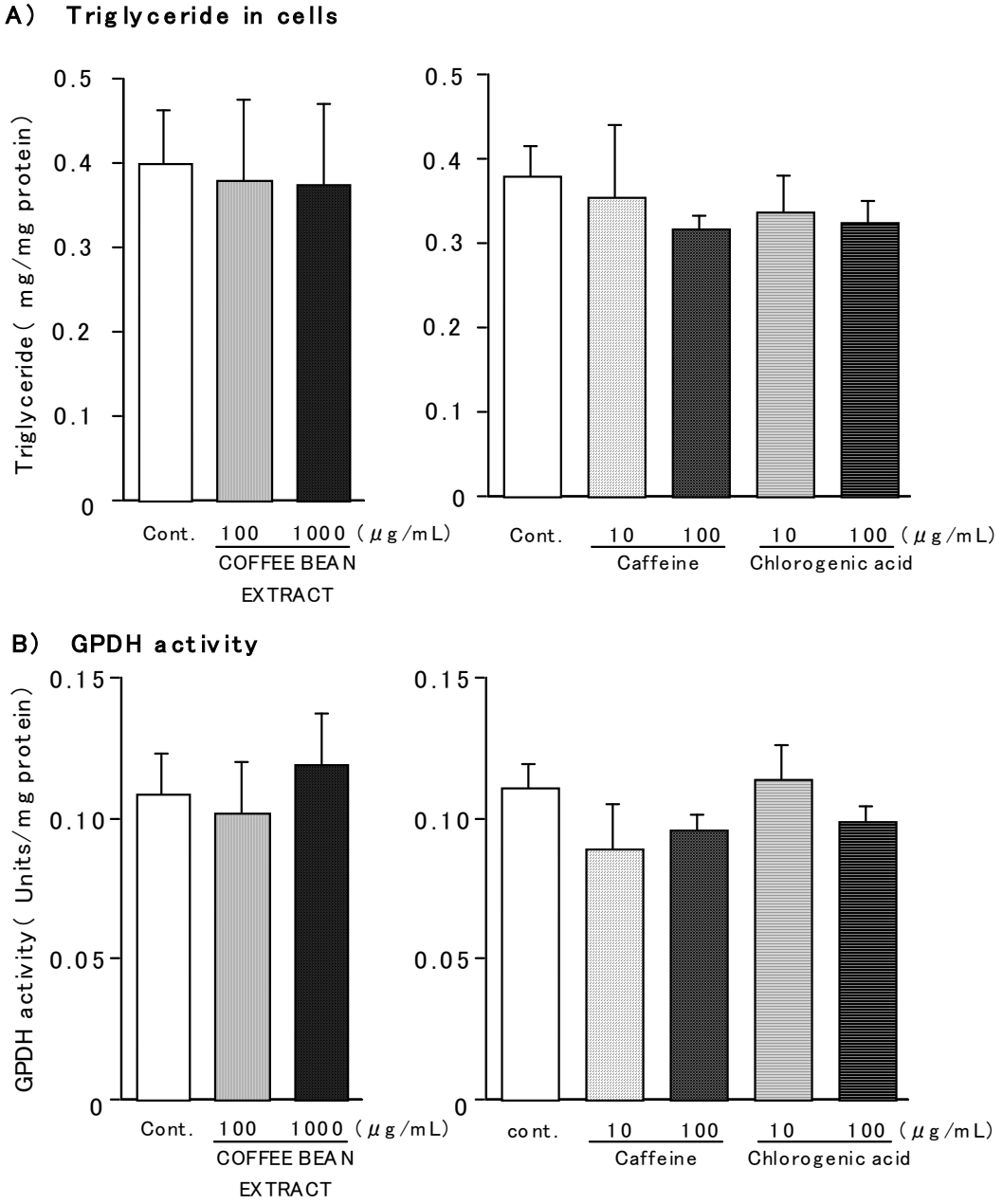


Fig. 5. Effects of COFFEE BEAN EXTRACT and its constituents on differentiation of 3T3-L1 adipocytes.
(n=6-7, mean±S.E.)

Materials and Method:

Experimental design and treatment. 3T3-L1 adipocytes ($5x10^4$ cells/ml) were cultured in DMEM medium (high glucose) supplemented with 10% fetal calf serum. Cells differentiation was induced by medium replacement containing insulin (1 μg/ml), dexamethasone (0.25 μM) and isobutyl-methyxanthine (0.5 mM). 2 days later, the medium containing Coffee Bean Extract and its functional components and insulin (1μg/ml) was introduced. The new medium was cultured for 6 days with replacement

every alternate day. Differentiation of adipocytes and cell triglyceride levels were determined. Cell differentiation was determined using GPDH (glycerol 3-phosphate dehydrogenase) as biochemical marker.

b. Inhibits fat accumulation and prevents weight gain (*in vivo*)

The effect of Coffee Bean Extract was examined *in vivo*. Its effect on body weight was assessed and compared with roasted coffee bean extract simultaneously. Findings indicated that Coffee Bean Extract effectively inhibited weight gain confirming the inhibitory effect of caffeine & chlorogenic acid on weight gain (as shown in Fig. 6). Coffee Bean Extract (raw coffee bean extract) demonstrated superior suppressive effect on weight gain upon comparison with roasted coffee bean extract.

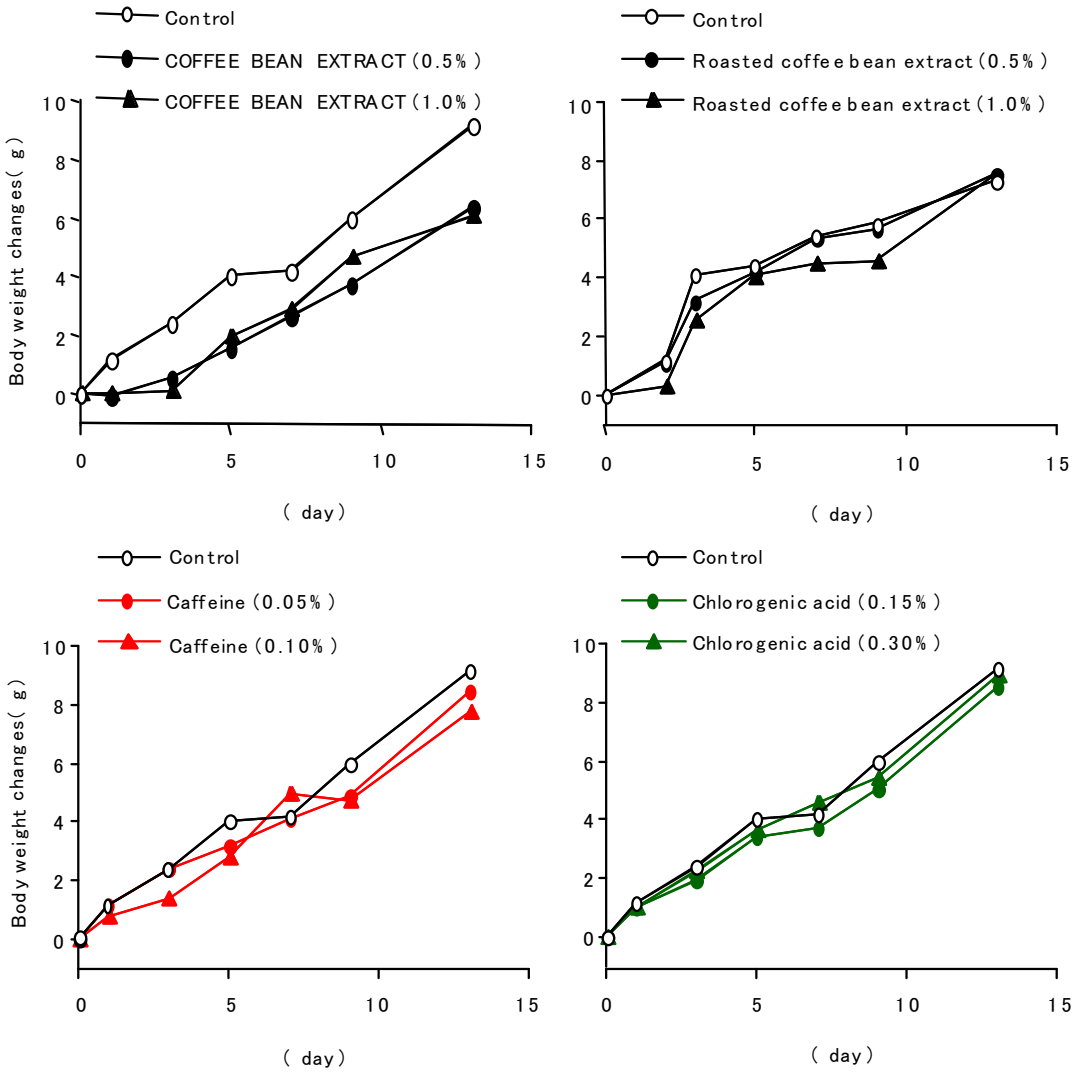


Fig. 6. Effects of COFFEE BEAN EXTRACT, roasted coffee bean extract and its constituents on mice body weight.

In addition, the effect of coffee bean extract and its functional constituents on visceral fat accumulation was assessed. Again, results confirmed that Coffee Bean Extract inhibited fat accumulation in the epididymis and perirenal area (Fig. 7).

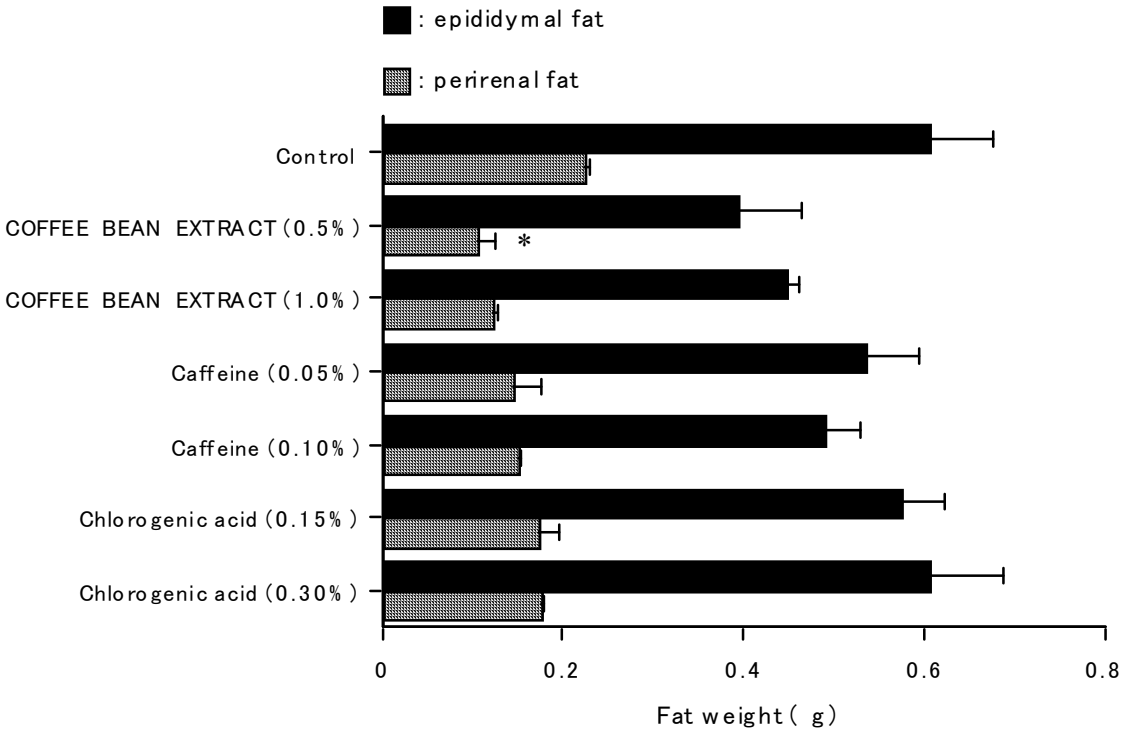


Fig. 7. Effects of COFFEE BEAN EXTRACT and its constituents on mice visceral fat accumulation.

[n=7, mean ± S.E., *: $p<0.05$ (Dunnett's multiple range test)]

Materials and methods:

Experimental design and treatment. 6 weeks old male ddY mice had free access to food (by CE-2, Clea Japan Inc.) for 13 days. Food was fortified with coffee bean extract (0.5% & 1%), caffeine (0.05% & 0.1%), and chlorogenic acid (0.15% & 0.3%). Weight of mice was measured at 2-day intervals while weight of visceral fat was measured at the end of experiment.

c. Prevents fatty liver (*in vivo*)

The effect of coffee bean extract, chlorogenic acid and caffeine on liver fat was examined. Liver triglyceride and cholesterol level were measured as marker for fatty liver. As illustrated in Fig. 8, coffee bean extract and chlorogenic acid significantly reduce liver triglyceride level. However, total liver cholesterol level remained unchanged.

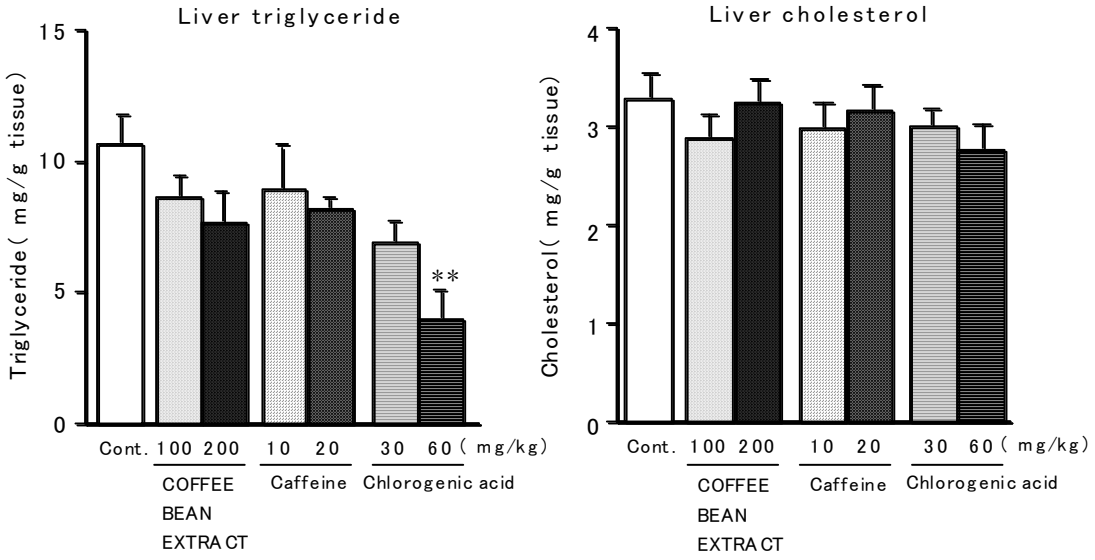


Fig. 8. Effects of COFFEE BEAN EXTRACT and its constituents on mice liver triglyceride and cholesterol level.
[n=6, mean±S.E., **: p<0.01 (Dunnett's multiple range test)]

Material and method:

Animals and treatments. 24 male ddY mice, 5 weeks old, were breeded and maintained for 1 week and divided into 7 groups. Mice livers were removed at the end of experiment (without prior fasting).
Sample collection and assays. Samples containing coffee bean extract in 5%w/v gum Arabic suspension were orally administered to mice once daily for 2 weeks. Liver triglyceride and cholesterol level were determined by a measuring kit (from Wako Pure Chemicals Industries Co., Ltd.)

d. The effect of Coffee Bean Extract on weight gain and fat accumulation in mice fed with high fat diet (*in vivo*)

The suppressive effect of Coffee Bean Extract on body weight was further assessed in mice fed with high fat diet. A significant suppressive effect was observed in mice treated with 0.5% and 1% Coffee Bean Extract (Fig. 9.). At both concentrations, the suppressive effect of Coffee Bean Extract on body weight was greater than mice on low-fat diet. In the experiment, the amount of diet intake was reduced in mice fed with 1% Coffee Bean Extract, whereas the amount of intake was higher in mice fed with

0.5% Coffee Bean Extract. The amount of diet intake and changes in body weight of high-fat diet mice is shown in Table 1.

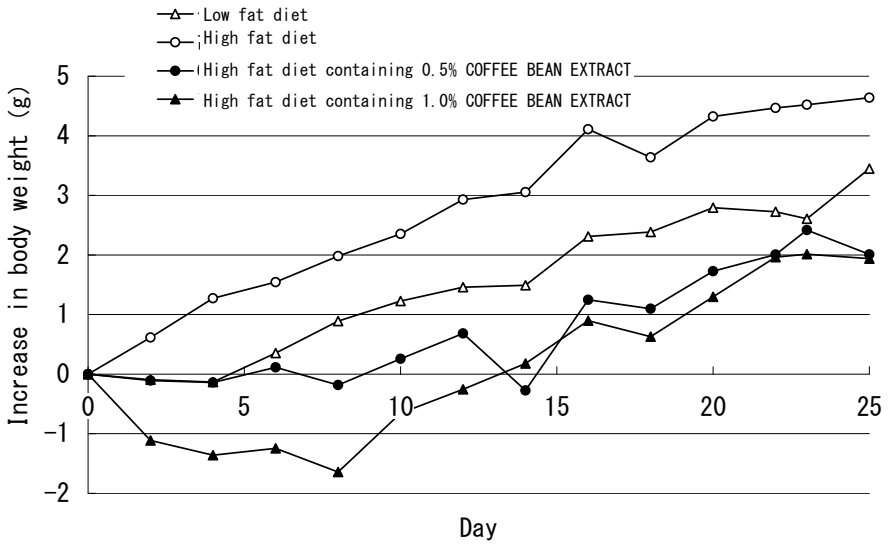


Fig. 9. The effect of Coffee Bean Extract on body weight and fat accumulation of mice fed with high fat diet.（n=6）

Table 1. Body weight and amount of diet intake.

	Initial body weight (g)	Final body weight (g)	Increase in body weight (g)	Amount of diet intake (g)
Low fat diet	20.6±0.2	24.1±0.7	3.5±0.6	95.3
High fat diet (HF)	21.0±0.8	25.7±0.6	4.6±0.4	65.5
HF+ 0.5% extract	23.6±0.5	25.6±0.3	2.0±0.7**	67.4
HF+ 1.0% extract	21.3±0.2	23.3±0.2*	1.9±0.2**	57.9

[n=6, mean ± SE, Significant differences *vs* HF, *：p<0.05, **：p<0.01 (Dunnett's multiple range test)]

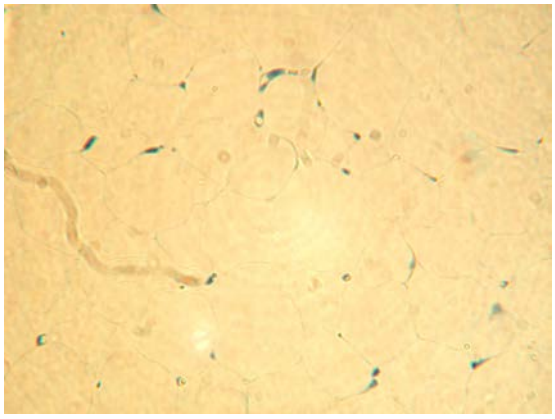
There was an increase in epididymal fat in mice fed with high fat diet (Table 2). Coffee Bean Extract demonstrated a dose-dependent effect on the prevention of fat accumulation in mice fed with high fat diet. The absolute liver weight and relative liver weight was significantly reduced in mice fed with 0.5% Coffee Bean Extract.

Table 2. Epididymal fats and liver weight in mice.

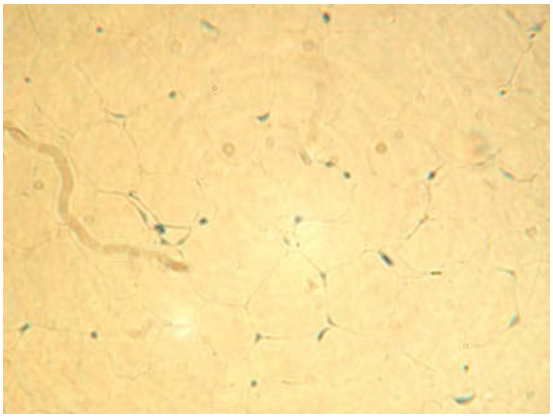
	Epididymal fat		Liver	
	(g)	(mg/g b. w.)	(g)	(mg/g b. w.)
Low fat diet	0.30±0.04	12.6±1.5	1.22±0.04	50.7±1.0
High fat diet (HF)	0.52±0.05	20.1±1.7	1.13±0.04	44.0±0.8
HF+ 0.5% extract	0.36±0.07	14.1±2.6	0.95±0.07*	37.1±2.3**
HF+ 1.0% extract	0.26±0.02**	11.3±0.8**	1.00±0.02	43.0±0.9

[n=6, mean ± SE, Significant differences *vs* HF, *：p<0.05, **：p<0.01 (Dunnett's multiple range test)]

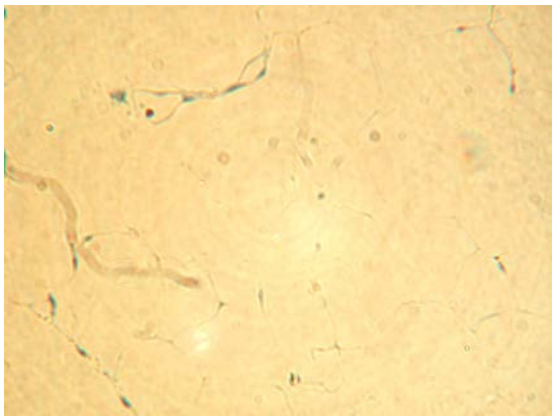
Fig. 10 & 11 are microscopic illustrations of epididymal fat and hepatic slice specimens of mice fed with high-fat diet respectively. In Fig.11, cell membrane is stained and the size of cell is comparable to that of low fat diet. Cell of high-fat diet mice is usually larger. However, size of cells in high-fat mice fed with Coffee Bean Extract 05% and 1% is smaller than low-fat diet mice.



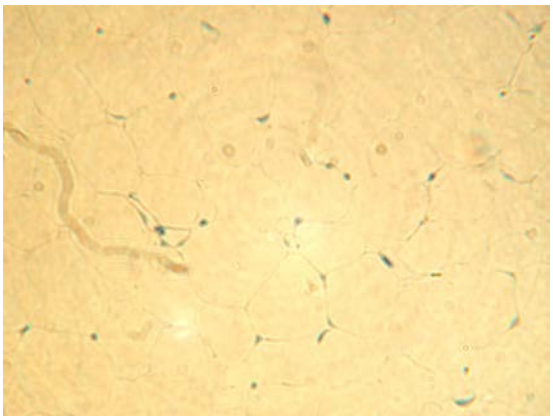
Low fat diet



HF+ 0.5% extract



High fat diet (HF)



HF+ 1% extract

Fig. 10. Microscopic illustrations of epididymal fat (Toluidin blue staining, x100)

Hepatocyte membrane in mice fed with high fat diet mice is usually invisible. Whereas hepatocyte membrane in high-fat diet mice supplemented with Coffee Bean Extract is clearly observed with large amount of visible glycogen granules (as shown in Fig. 11). Hence, Coffee bean extract possess inhibitory/suppressive effect against weight gain and fat accumulation in high-fat diet group.

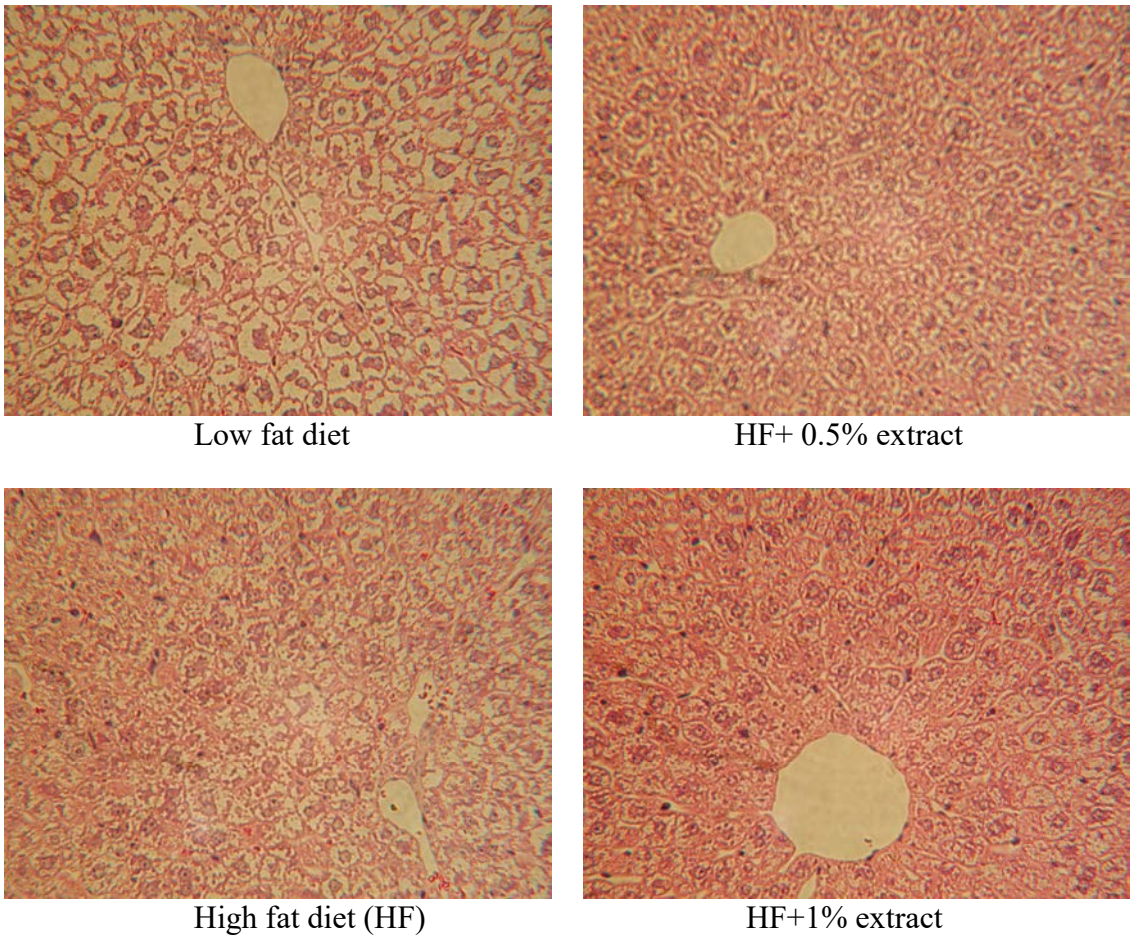


Fig. 11. microscopic illustrations of hepatocytes (H. E. staining, x100)

Materials and methods:

Experimental design and treatment. 24 male C57BL/6J mice, 7-wk old were divided into 4 groups and breeded for 2 weeks. Low fat diet (containing corn oil 5%, casein 20%, cellulose 4%, Harper mineral mix 3.5%, Harper vitamin mix 1% & corn starch 66.5%) was given to group 1. Meanwhile, group 2-4 were fed high-fat diet supplemented with Coffee Bean Extract 0.5% and 1%. Duration of experiment was 25 days. Body weight and amount of food intake was measured prior to and after administration of diets. At the end of experiment, blood samples were collected, epididymal fats and livers were removed accordingly.

e. The effects of Coffee Bean Extract on body weight and fat synthesis and metabolism in rats fed with cholesterol diet *(in vivo*)

Professor Kazunari Tanaka and coworkers in Siebold University of Nagasaki evaluated the effect of Coffee bean extract on rats fed the cholesterol diet for 4 weeks. As shown in Table 3, Coffee bean extract significantly suppressed increase in body weight, liver weight and amount of visceral fats. Serum and liver triglyceride dramatically reduced. Moreover, the activity of liver fatty acid synthase and malic

enzyme were suppressed and the activity of carnitine palmitoyltransferase, a rate-limiting enzyme of β-oxidation, was enhanced. These result shows that coffee bean extract reduce the abdominal fat and liver triglyceride by improving fat metabolism in liver.

Table 3. Effects of coffee bean extract on rat obesity parameters fed cholesterol diet.

	Control	Coffee bean extract
＜Organ weight＞		
Initial body weight（g）	143±2	142±3
Final body weight（g）	**386±10**	**303±14***
Body weight gain（g）	**244±9**	**161±12***
Food intake（g）	22.7±0.5	21.5±1.0
Food efficiency（g gain/g intake）	0.40±0.01	0.28±0.02*
Liver weight（g）	**24.5±1.0**	**18.2±1.5***
(g/100 g 体重)	6.33±0.13	5.94±0.28
Adipose tissue		
Perirenal (g)	**6.78±0.87**	**2.00±0.34***
Epididydimal (g)	**5.01±0.54**	**2.53±0.28***
Relative adipose tissue weight		
Perirenal（g/100g weight）	**1.74±0.20**	**0.65±0.09***
Epididydimal（g/100g weight）	**1.28±0.11**	**0.82±0.06***
＜Liver lipid＞		
Triglyceride (mg/dL)	**75.6±6.7**	**44.0±3.2***
Phospholipid (mg/dL)	**27.7±0.5**	**31.3±1.0***
＜Serum parameters＞		
Triglyceride（mg/dL）	**180.3±31.6**	**66.1±11.7***
Phospholipid（mg/dL）	**184±13**	**193±9**
LPO（nmol/mL）	**15.6±1.0**	**11.8±0.5***
SOD（%）	16.5±0.9	15.4±0.6
＜Fatty acid metabolizing enzymes (nmol/min/mg protein)＞		
Fatty acid synthase	**4.88±0.84**	**2.94±1.17***
Carnitine palmitoyltransferase	4.12±0.37	4.82±0.34
Malic enzyme	**19.3±0.84**	**13.8±1.49***
Glucose-6-phosphate dehydrogenase	15.9±2.20	12.0±1.33
Phosphatidic acid phoshohydrolase（mic）		
Mg+	**4.34±0.20**	**5.56±0.42***
Mg-	2.94±0.23	3.06±0.13

Mean ± SE, n=6, *: $p<0.05$

Experimental design and treatment. Male SD rats (4 weeks old) were fed the diet (AIN-93 composition) containing 1% coffee bean extract, 0.5% cholesterol and 0.125% sodium cholate for 4 weeks.

(3) Stimulation of lipolysis

a. Lipolytic effect (*in vitro*)

Caffeine is known to activate hormone sensitive hepatic lipase activity in fat cells to promote the breakdown of fat. The lipolytic effect of Coffee Bean Extract and its functional component were examined and compared with commercially available weight loss products. As illustrated in Fig.12 Coffee Bean Extract demonstrated

superior lipolytic effect on comparison with citrus extract containing 30% syneprine, however, its effectiveness was comparable to synthetic capsaicin and synephrine. Findings suggested that caffeine is responsible for the lipolytic action in Coffee Bean Extract. Meanwhile, lipolytic effect was observed in coleus forskohlii extract. Study conducted by *Tholon et al*. demonstrated the lipolytic action of caffeine in commercially available topical slimming preparations. Coffee bean extract is suitable to be incorporated into cosmeceutical preparations for slimming and prevention against the unsightly appearance of cellulite.

*) Tholon L, *et al*., An *in vitro*, *ex vivo*, and *in vivo* demonstration of the lipolytic effect of slimming liposomes: An unexpected alpha(2)-adrenergic antagonism. *J. Cosmet. Sci*. **53**, 209-18 (2002).

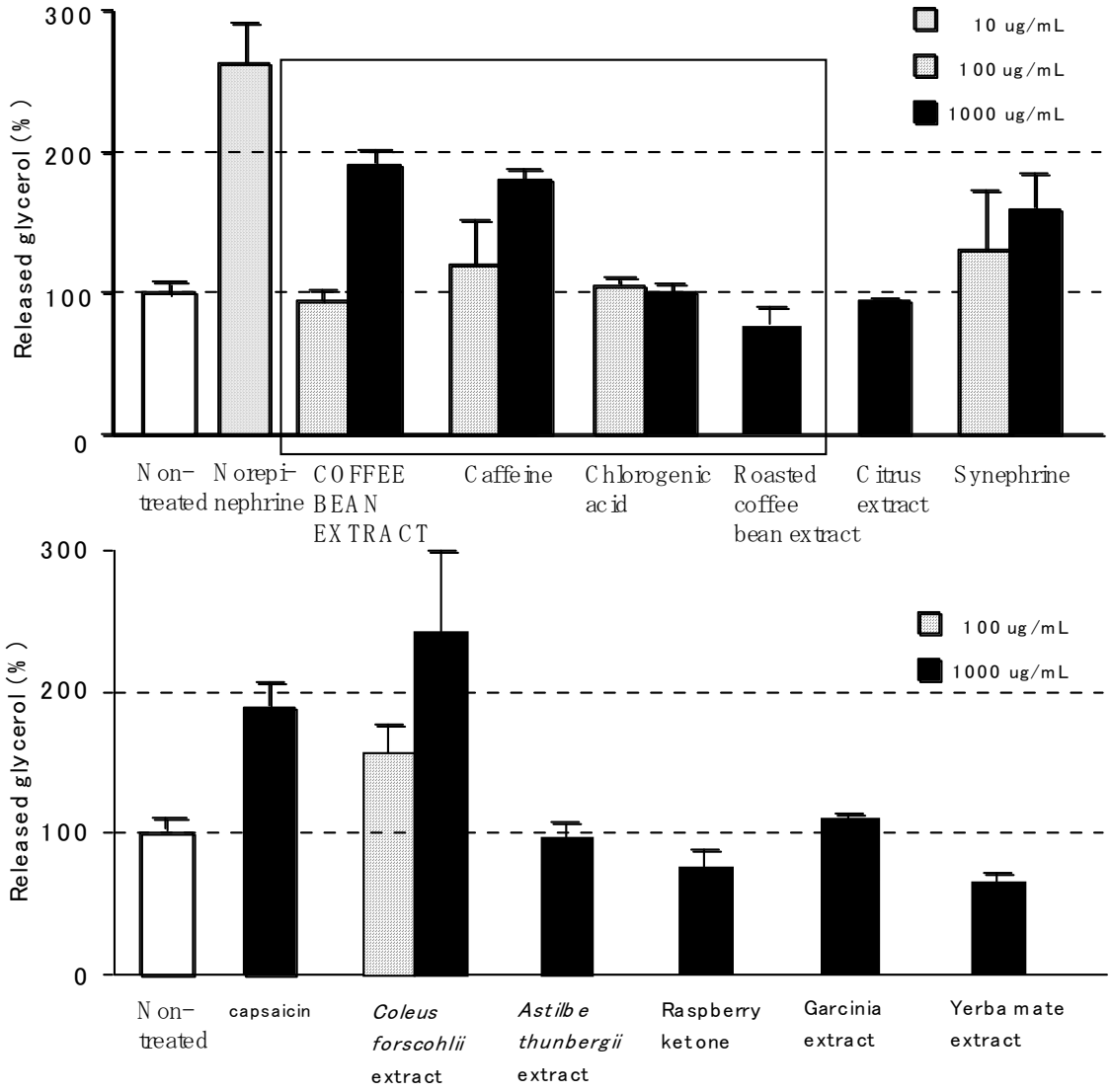


Fig. 12. Effects of COFFEE BEAN EXTRACT, its constituents and commercially available weight loss ingredients on glycerol release from rat epididymal fat. (n=4, mean±S.E.)

Materials and Methods:

Experimental design and treatments. Epididymal fat of male Wistar rats was removed and incubated in Medium 199 containing test sample. Incubation was maintained at 37ºC for 3 hours. After incubation, fat was removed and glycerol concentration was measured using F-kit Glycerol (Roche Japan Co., Ltd.)

b. Reduces serum triglyceride (*in vivo*)

Further *in vivo* study was prompted to confirm the lipolytic effect of coffee bean extract. The effect of coffee bean extract, chlorogenic acid and caffeine on serum triglyceride level was assessed. As shown in Fig. 13, Coffee Bean Extract and caffeine demonstrated a marked reduction in blood triglyceride level similar to that of capsaicin. On the other hand, the triglyceride lowering effect of chlorogenic acid was comparable to that of synephrine.

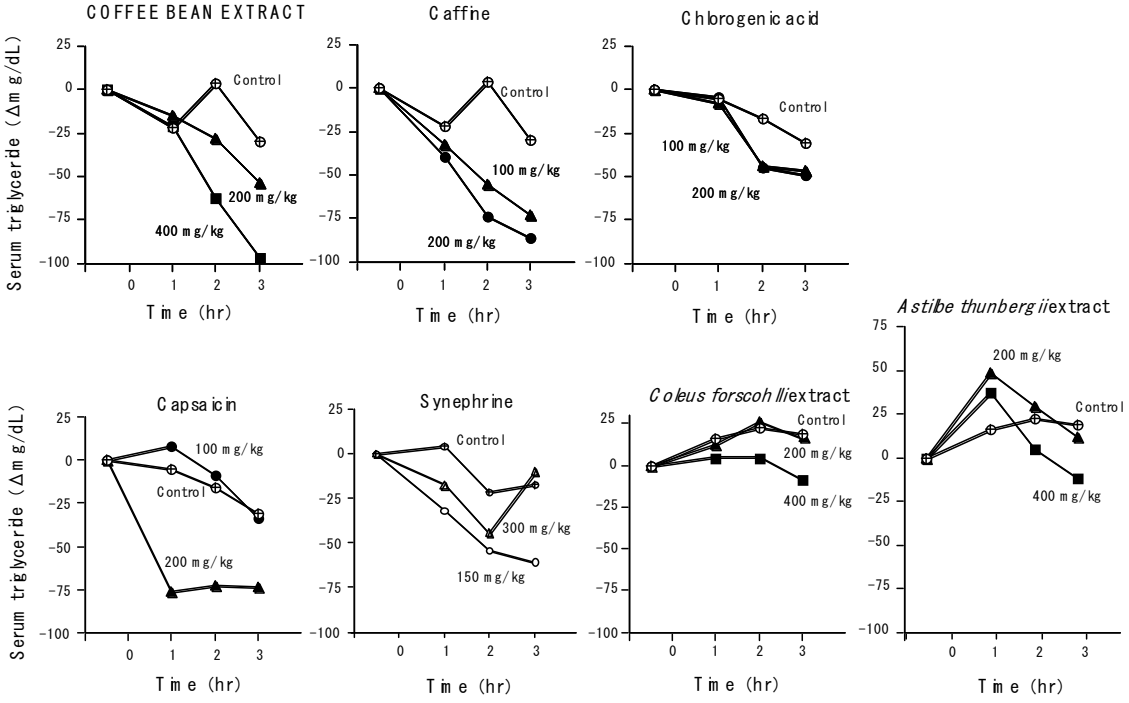


Fig. 13. Effects of COFFEE BEAN EXTRACT, its constituents and commercially available weight loss ingredients on serum triglyceride in mice.

Materials and Methods:

Animals and treatment. 6-wk old male ddY mice were fasted. Blood sample was collected for initial reading. 30 minutes later, 5%w/v Arabic gum containing samples (10 ml/kg) of Coffee Bean Extract, caffeine, chlorogenic acid, capsaicin and synephrine was given orally. Blood samples were collected every hour and serum triglyceride was

measured.

(4) Promotion of fat metabolism

a. Enhances uncoupling (UCP) protein in mouse brown adipocytes (*in vivo*, citation)

Brown adipose tissue is responsible for the regulation of body temperature and energy production through utilization of excess caloric intake. Brown fat uncoupling protein (e.g. UCP-1) plays an important role in fuel metabolism.

Reviews showed that caffeine promote UCP-1 expression in brown adipose tissue of diabetic mice (as illustrated in Fig.14) Hence, caffeine promotes fat metabolism through the release of energy for temperature regulation.

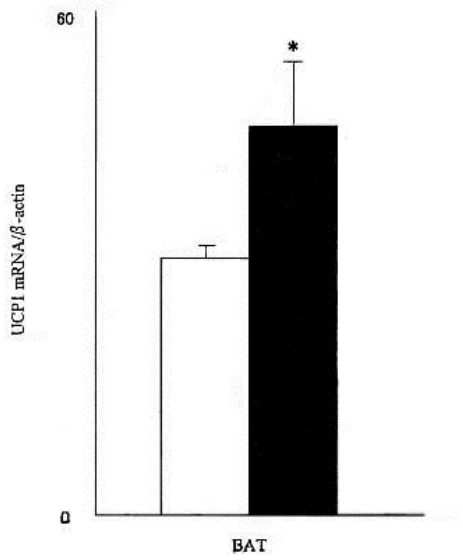
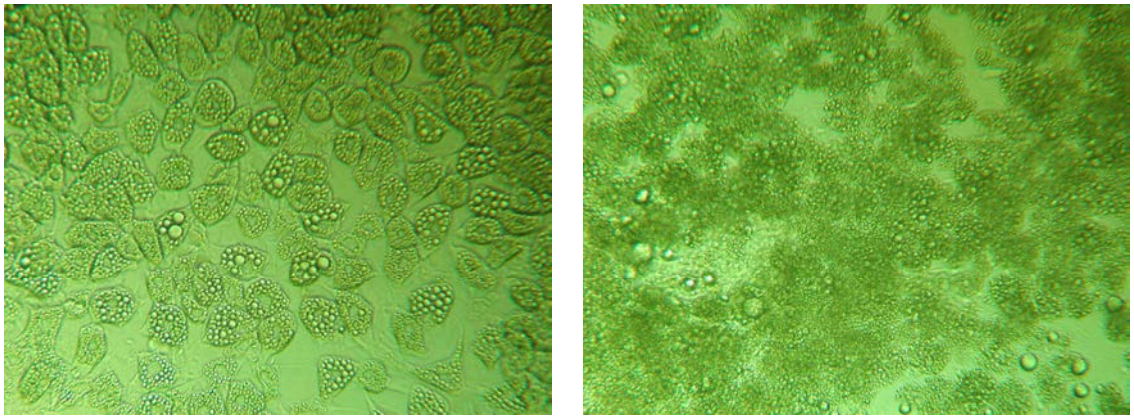


Fig. 14. Enhancement of caffeine on uncoupling protein 1(UCP-1) expression in KK mouse brown adipose tissue (BAT).
Physiological saline (□) & caffeine (60 mg/kg, ■) was administered subcutaneously to mice. Inter-scapular brown adipose tissue was collected 4 hours later. The UCP-1 mRNA expression level was evaluated by RT-PCR and expressed as the ratio to β-actin as a marker (n = 15, mean ± S.E., *:$p < 0.05$).

Ref. Kogure A., *et al.*, Effects of caffeine on the uncoupling protein family in obese yellow KK mice. *Clin. Exp. Pharmacol. Physiol.* **29**, 391-4 (2002).

b. The effect of Coffee Bean Extract on oil vesicles of brown adipocytes (*in vitro*)

The effect of Coffee Bean Extract on brown adipocytes and its oil vesicles were examined *in vitro*. It was found that, that oil vesicles within brown adipocytes was significantly smaller after treatment with coffee bean extract at concentration (1000µg/ml), as illustrated in Fig. 15.



Control COFFEE BEAN EXTRACT (1000 µg/ml)

Fig. 15. Effects of Coffee Bean Extract on brown adipocytes.

Materials and Methods:

Experimental design and treatment. Commercially available brown adipocytes from rats (by Hokudo Co.) were used. Pre-adipocytes isolated from rat epididymal fat tissue was cultured for 6 days to induce production of brown adipocytes. Initial size of oil vesicles was observed, coffee bean extract at concentration 1000 µg/ml was added and cells were incubated for 18 hours. Size of oil vesicles was observed again after treatment with Coffee Bean Extract.

c. Enhances of liver mitochondrial carnitine palmitoyl transferase (CPT) activity (*in vivo*)

Liver fat metabolism occurs in hepatocytes where fat is metabolized in a process called β-oxidation. Carnitine and carnitine palmitoyl transferase (CPT) is the rate limiting enzyme responsible for β-oxidation. The effect of Coffee Bean Extract on fat metabolism (*in vivo*) was examined. Sesamin was used as a positive control. As illustrated in Fig. 16, Coffee Bean Extract demonstrated a dose-dependent enhancement of carnitine palmitoyl transferase (CPT) activity, thus promotes fat metabolism. Although chlorogenic acid and caffeine did not affect on CPT activity, neochlorogenic acid (3-caffeoylquinic acid) and feruloylquinic acid mixture (4- and 5 feruloylquinic acids) enhanced CPT activity. These compounds were found to be CPT activity enhancers in Coffee Bean Extract.

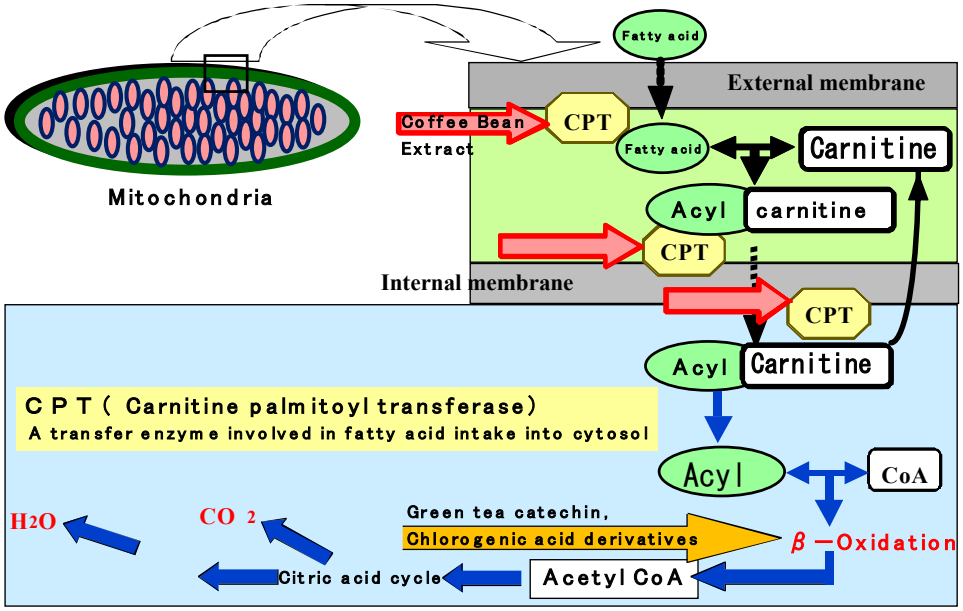


Fig. 16. Pathway of liver fat metabolism & site of action of Coffee Bean Extract & other weight loss promoting ingredients.

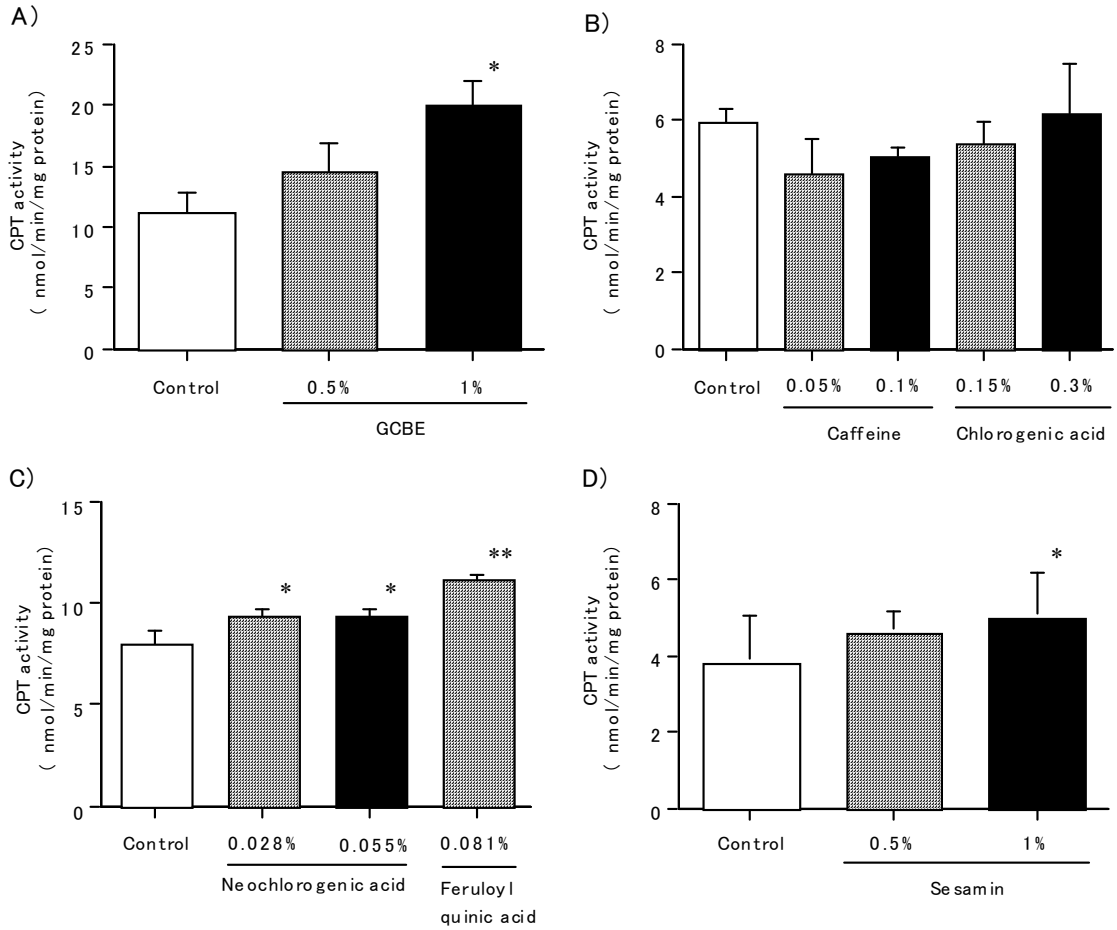


Fig. 17. Effect of COFFEE BEAN EXTRACT and its constituents on mitochondrial CPT activity in mice.
(n=4-5, mean±S.E., *: $p<0.05$)

Materials and methods:

Animals and treatments. 7-wk old male ddY mice had free access to food (CE-2, Clea Japan Inc.) containing sample for 6 days. Cervical spine was dislocated and liver of mice were removed and homogenized.
Sample collection and assays. 0.25 M sucrose at weight 6x that of liver and Tris buffer (pH 7.4) containing 1mM EDTA was used for homogenization. The homogenized solution was centrifuged at 3,000 rpm for 10 minutes. The supernatant was centrifuged again at 11,000rpm for 10 minutes. Mitochondrial fraction was obtained as sediments and further suspended in Tris buffer (2.5ml) CPT activity was measured using DTNB method. *
*) Markwell M. A. K. *et al*., The subcellular distribution of carnitine acyltransferases in mammalian liver and kidney. *J. Biol. Chem*., **248**, 3426-32 (1973)

(5) Prevention of diabetes mellitus
a. Delays sugar absorption (*in vivo*)

The effect of coffee bean extract on post-prandial blood sugar level was assessed. As illustrated in Fig. 18, 400 mg/kg of Coffee bean extract effectively delayed elevated post-prandial blood sugar level. In addition, coffee bean extract at 200 mg/kg effectively suppressed elevated post-prandial glucose in sucrose loading mice.

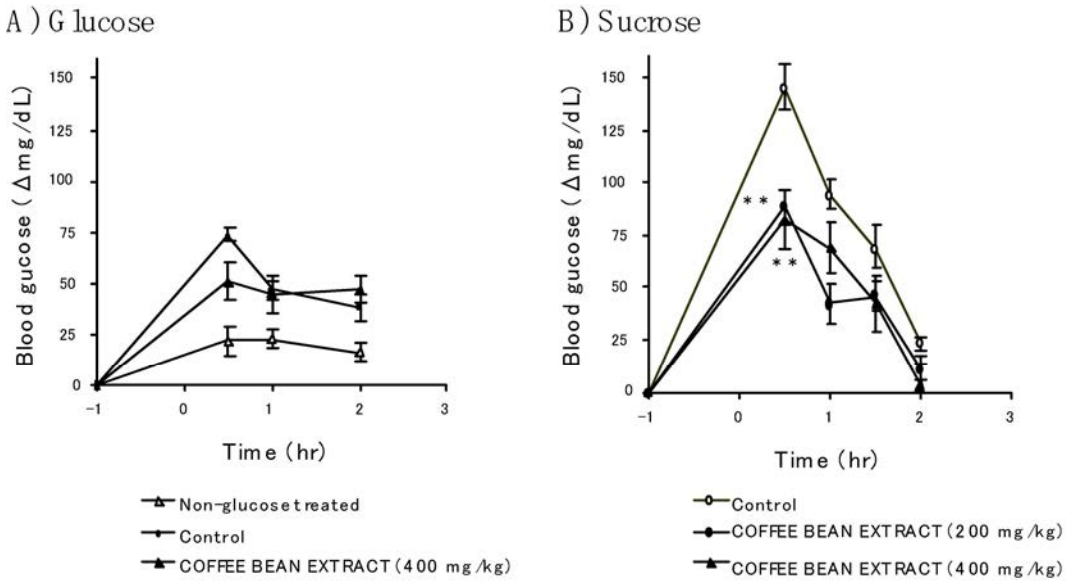


Fig. 18. Effect of COFFEE BEAN EXTRACT on elevated serum glucose level in glucose- or sucrose-loaded mice. (n=6, mean±S.E.,)

Materials and methods:
Animals and treatment. 6-wk old ddY mice were fasted for 18 hours and blood samples were collected. Solution (10 ml/kg) containing Coffee Bean Extract was given orally. Glucose loading followed 1 hour later.
Sample collections and assays. Loading of glucose was given at 0.5g/kg. Blood samples were collected after glucose loading at 0.5, 1 and 2 hours. Serum was separated

prior to determination of glucose concentration using enzymatic method, Determiner GL-E, Kyowa Medics Co., Ltd.)

b. Inhibits activity of α-glucosidase (*in vitro*)

The effect of coffee bean extract on α-glucosidase, a rate-limiting enzyme in gluconeogenesis, was assessed. Experiments revealed that coffee bean extract produces relatively potent inhibitory effect against α-glucosidase but no inhibitory effect on α-amylase as illustrated in Fig. 19 and Table 4. Findings also revealed that the active components of coffee bean extract, chlorogenic acid and caffeic acid exhibited inhibitory effect on α-glucosidase.

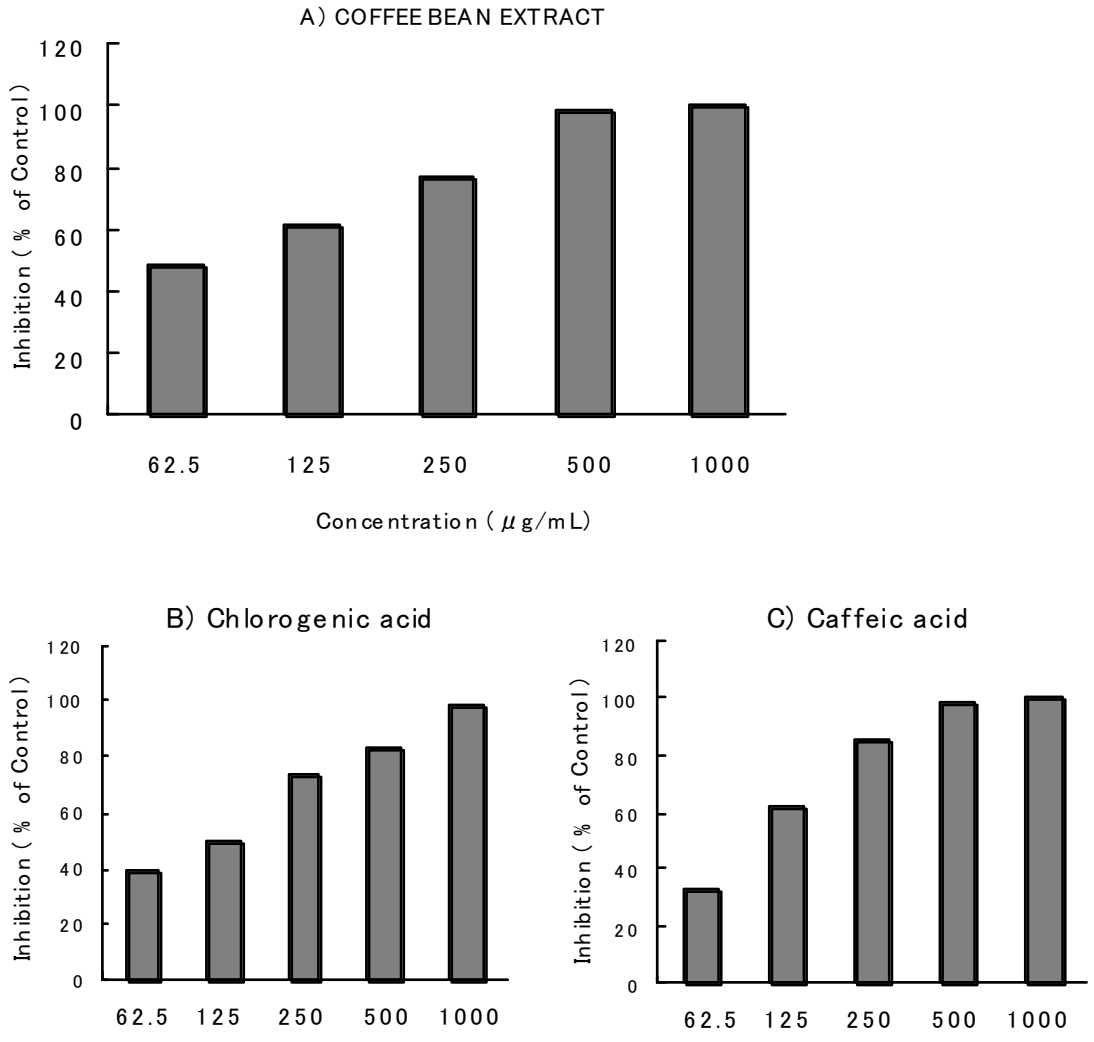


Fig. 19. Inhibitory effects of COFFEE BEAN EXTRACT and its constituents on α-glucosidase acitivty.

Table 4. α-Glucosidase inhibitory activities（IC_{50}）

	IC_{50} (μ g/mL)
COFFEE BEAN EXTRACT	70
Caffeine	>1000
Chlorogenic acid	100
Caffeic acid	100
Quinic acid	>1000

Materials and Methods:

Reagents. Powdered rat intestine was used as source of α-glucosidase.

Sample collections and assays. Powdered rat intestine was dissolved and centrifuged in 10 x 0.1M phosphate buffer (pH 7.0). Enzyme α-glucosidase recovered as the supernatant layer, treated with 0.2mM of 4-methyl-umberlliferyl-α-D-glucopyranoside. Samples of Coffee Bean Extract and its constituents were dissolved in DSMO and diluted x2 in 4% DSMO containing phosphate buffer solution. The diluted samples were placed in a microplate (50 μl/well) and substrate (25 μg/well) was added. The mixture was pre-incubated at 37ºC for 10 minutes. Enzyme was added and followed by further incubation for 30 minutes (final concentration of enzyme 1mg protein/ml; final concentration of substrate 0.05mM). The reaction was ceased by addition of 0.2M Na_2CO_3, activity was measured by fluorescene microplate reader at excitation wavelength 366 nm & emission wavelength 450 nm.

c. Inhibitory effect of chlorogenic acid on gluconeogenesis (*in vitro*, citation)

Study conducted by Arion W. J. *et al*., shown that the chlorogenic acid in coffee bean extract act as competitive inhibitor of hepatic glucose-6-phosphatase. The activity of glucose-6-phosphatase is normally high in patients with Type II diabetes mellitus. Gluconeogenesis is activated leading to hyperglycemia. As illustrated in Lineweaver-Burk plot in Fig.20, chlorogenic acid inhibits gluconeogenesis in Type II diabetes and under restriction of sugar intake.

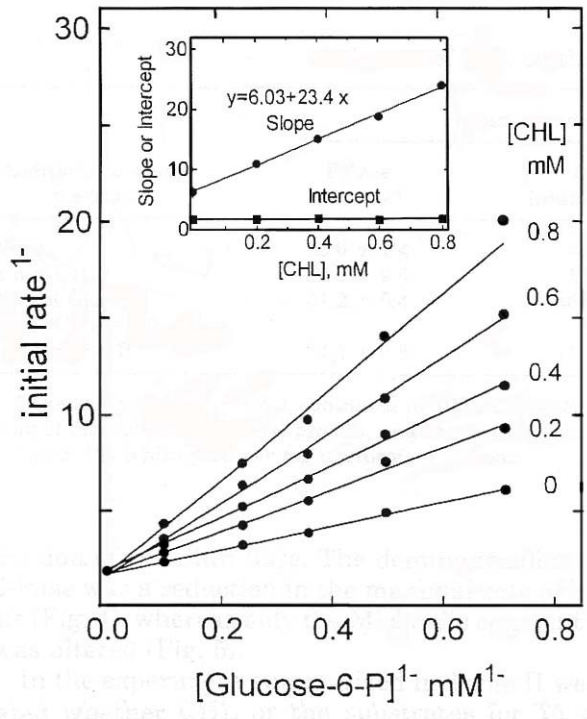


Fig. 20. An inhibitory plot of chlorogenic acid (CHL) against glucose-6-phosphatase (A Lineweaver-Burk plot).

In the graph outside, the vertical axis indicates enzyme activity, and the horizontal axis indicates the concentration (1-10mM) of the substrate (glucose-6-P). Since each plot (measurement at CHL concentrations of 0-0.8mM) converges on the Y-axis, chlorogenic acid has competitive inhibitory effects on glucose 6-phosphatase.

On the inner graph, the vertical axis indicates the slope of the plot or the Y-intercept, and the horizontal axis indicates the chlorogenic acid concentration. The constant Y-intercept and linear changes in the slope show competitive inhibition.

Ref. Arion W.J., *et al*. Chlorogenic acid and hydroxynitrobenzaldehyde:new inhibitors of hepatic glucose 6-phosphatase. *Arch. Biochem. Biophys.* **339**, 315-22 (1997).

(6) Anti-oxidative activity
SOD-like mechanism and DPPH radical scavenging activity

Oxidative stress is associated with various degenerative diseases in the modern society. Atherosclerosis and thickening of atheroma generated as results of oxidized LDL cholesterol by free radicals. The SOD-like mechanism and DPPH scavenging ability of Coffee Bean Extract was evaluated. As illustrated in Fig. 21, coffee bean extract possess potent anti-oxidative effects due to its high concentration of chlorogenic acid.

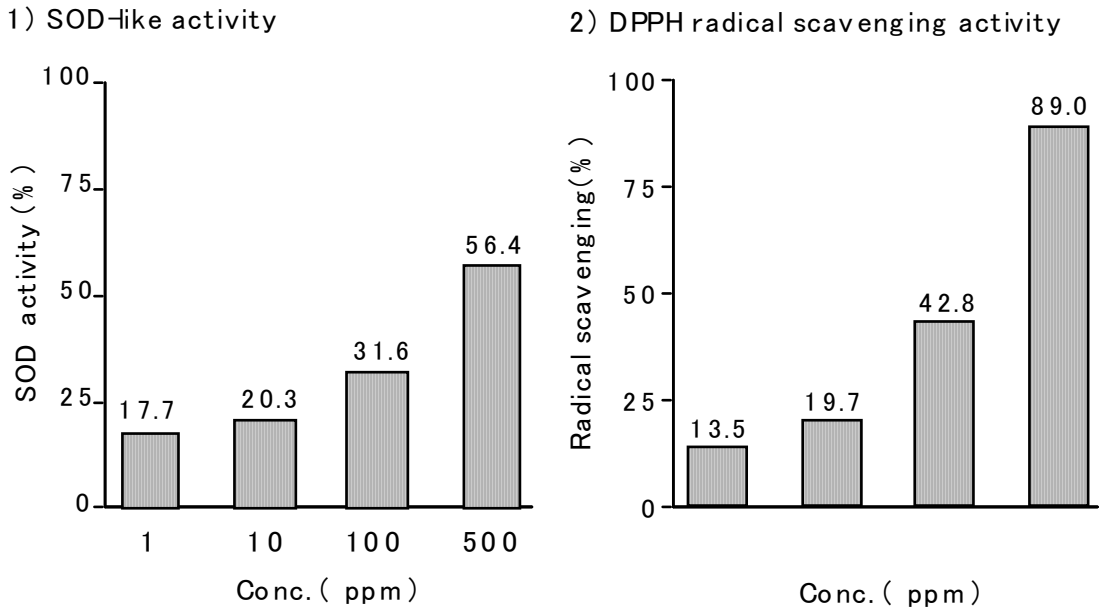


Fig. 21. Antioxidative activity of COFFEE BEAN EXTRACT.

***Materials and methods*:**
Antioxidative effect was determined using SOD Test Wako from Wako Pure Chemical Industries Co., Ltd. and DPPH reagent.

5. Human Studies

The effect of Coffee Bean Extract on human subjects was conducted. Normal, healthy male subjects were selected for a 4-week study. The effects of Coffee Bean Extract on body weight, body fat and blood profile was assessed.

***Materials*:** Coffee Bean Extract (Lot K320, 100 mg) containing chlorogenic acid 28.4%, chlorogenic acids related compound 48.7% & caffeine 12.5% in hard capsules were orally administered to study subjects.

Subjects: Six normal, healthy male subjects at age range 23-59 were selected. The average age of the study is 39.5 years old.

Methods: Subjects are required to fast prior to the study. Initial measurements on physical statistics e.g., body weight, height, body fat, impedance, waist circumference, hip circumference and thickness of abdominal fat were recorded.
Indications of BMI value:
Obese: BMI > 25
Standard: 18 < BMI < 25
Under weight: BMI < 20

Obesity ratio is determined according to the following calculations:

BMI (kg/m^2) = body weight (kg) / height (m) / height (m)

Obesity Ratio (%) = [(body weight – standard body weight)/standard body weight] x 100

Subjects were given Coffee Bean Extract 200 mg/day (2 capsules /day) for 4 weeks. No specific instruction prior administration, subjects were allowed to consume the capsule at anytime of the day. At end of study, measurements on physical statistics were recorded. Measurements of the physical statistics were compared and studied. Parametric t-test was used for assessment when $p < 0.15$ is significant.

Results and discussions

As shown in Table 5. Coffee Bean Extract 200mg/day reduces body weight, percentage of body fat, impedance, obesity ratio, waist circumference and thickness of abdominal fat. Changes on physical statistics of test subjects are shown in Fig. 22. It was clearly illustrated that Coffee Bean Extract produces a significant reduction in hip circumference. Meanwhile, body weight, obesity ratio, hip circumference and thickness of abdominal fat were reduced in 67% of the subjects. 50% shown decline in percentage of body fat, BMI value and impedance value.

The average changes on blood profile of test subjects were tabulated in Table 4. Blood triglyceride and free fatty acids level were reduced after consumption of coffee bean extract. In particular the blood triglyceride level has reduced from 292mg/dl to 205mg/dl during the study period.

As for blood sugar level, there was a decline in blood sugar level in 67% of subjects. However, there was an increase in the total cholesterol and creatinine level in 50% and 80% of the subjects respectively. (p=0.13, 0.05 respectively)

This study concluded that Coffee Bean Extract reduces body weight, body fat and blood triglyceride levels. The blood sugar, triglyceride and free fatty acids lowering effects of Coffee Bean Extract are contributed from inhibition of gluconeogenesis and lipolysis by chlorogenic acid and caffeine respectively. Reduction of blood parameters are also believed to be contributed by fat metabolism from caffeine and chlorogenic acid related compounds.

Meanwhile, increase in creatinine level was mainly due to increase in muscle which leads to a decrease in the impedance value.

Table 5. Physical sttistics and blood parameters of test subjects before and after Coffee Bean Extract (200 mg/day) ingestion.

	Before	After
Body weight（kg）	**64.2±5.6**	**64.1±4.8**
Percentage of body fat（%）	**19.7±3.0**	**19.4±4.2**
BMI（kg/m^2）	22.4±2.4	22.5±2.3
Impedance（Ω）	**482±64**	**475±46**
Fat ratio（%）	**12.7±2.6**	**12.5±3.2**
Obesity ratio (%)	**2.4±11.2**	**2.2±10.2**
Waist circumference（cm）	**77.8±6.7**	**77.0±7.3**
Hip circumference（cm）	**93.4±5.7**	**91.2±4.2** p=0.12
Waist / hipratio	0.83±0.04	0.84±0.05
Thickness of abdominal fat（mm）	**15.9±5.1**	**12.4±5.7**
Blood sugar（mg/dL）	**89.7±9.0**	**85.0±10.6** p=0.13
Cholesterol（mg/dL）	190.5±15.8	198.0±20.7 p=0.15
Triglyceride（mg/dL）	**108.7±94.5**	**98.7±63.9**
Free fatty acid（mEq/L）	**0.34±0.11**	**0.26±0.11**
Creatinine（mg/dL）	**0.95±0.15**	**1.07±0.10** p=0.05
Total protein（g/dL）	7.13±0.16	7.18±0.34

N=6, Mean±S. E.

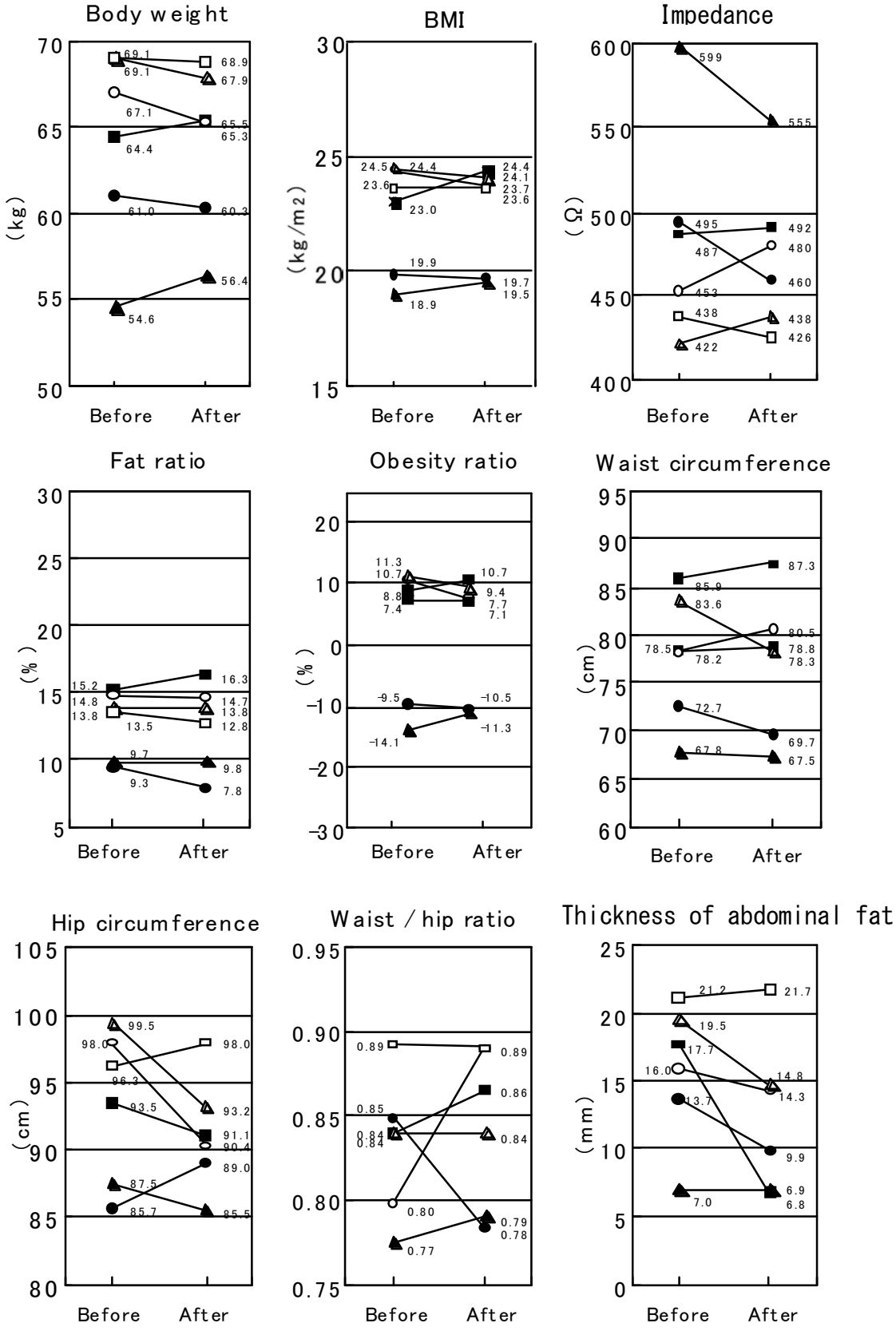


Fig. 22. Obese parameters in each subjects before and after Coffee Bean Extract (200 mg/day) ingestion.

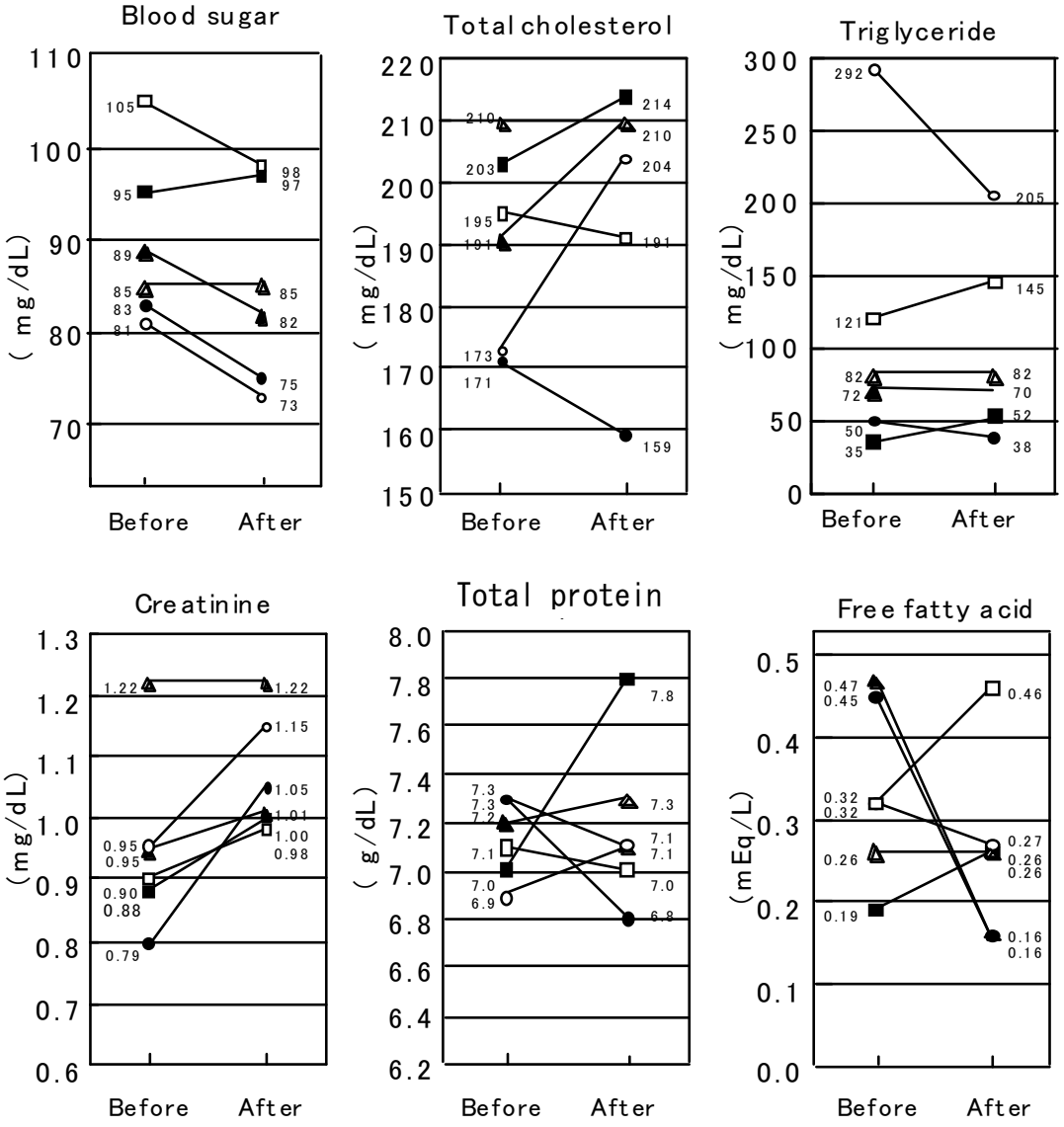


Fig. 23. Blood parameters in test subjects before and after Coffee Bean Extract (200 mg/day) ingestion.

6. Stability of COFFEE BEAN EXTRACT

(1) Thermal resistance

The content of chlorogenic acid and chlorogenic acids related compounds remained unchanged after heating at high temperature 120ºC for 1 hour (as illustrated in Fig. 25)

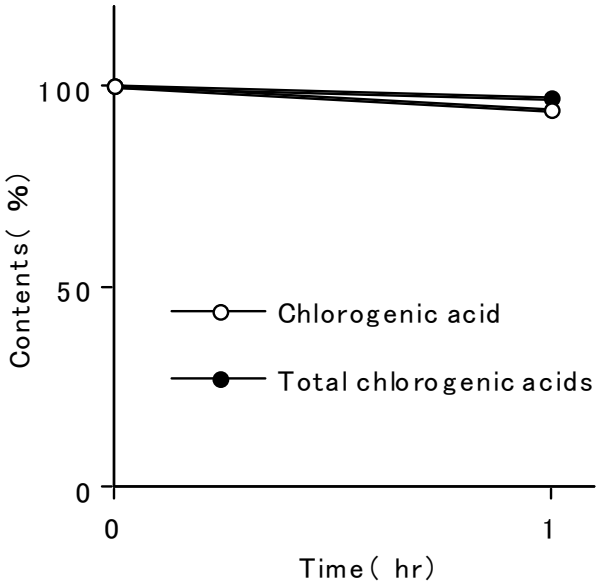


Fig. 24. The effect of heat on COFFEE BEAN EXTRACT (100% as initial value).

(2) pH stability

The content of chlorogenic acid and its related compounds in Coffee Bean Extract solution remained stable at pH range 3-9.

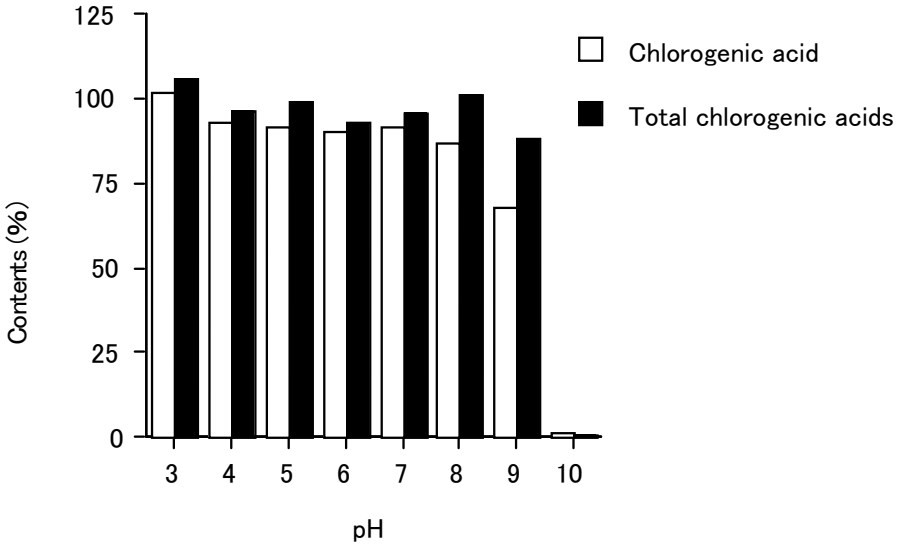


Fig. 25. Effect of pH on composition of chlorogenic acid and chlorogenic acids related

compounds (100% as initial value).

(3) Aqueous stability

Coffee Bean Extract in aqueous form was stored at room temperature and 4°C for 16 hours. No changes observed in its aqueous state at both neutral & acidic pH range. Coffee Bean Extract is highly stable in its aqueous state.

pH	Condition	
	Room temperature	4°C
Neutral (pH5-6)	Negative (up to 50%concentration)	Negative (up to 50% concentration)
Acid (pH3)	Negative (up to 50% concentration)	Negative (up to 50% concentration)

(4) Aqueous stability in beverages

Aqueous Coffee Bean Extract [(200 mg in 500 ml of water, branch-chained amino acid (BCAA) beverages (company A, B & C)) in PET bottle was kept under different conditions as stipulated in the graphs below for 6 months. The chlorogenic acid and its related compounds remain stable in water and BCAA beverages. Coffee Bean Extract, chlorogenic acid and chlorogenic acids related compound are highly stable and suitable for applications in beverages.

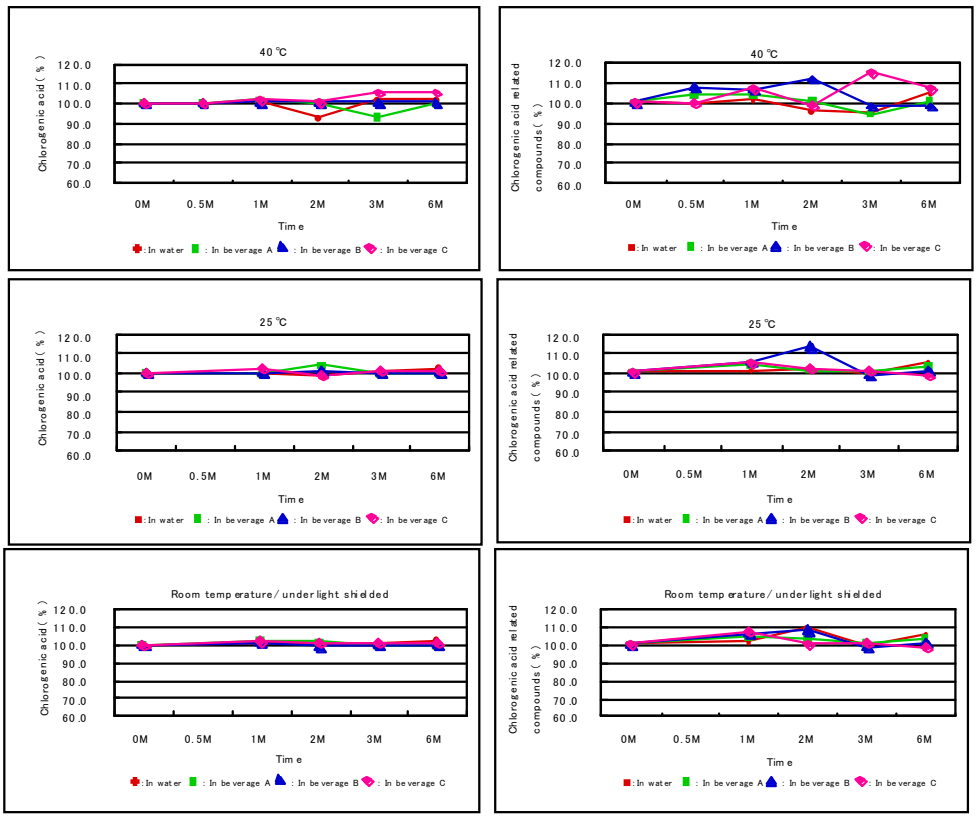


Fig. 26. Aqueous stability of chlorogenic acid and its derivatives in beverages.

7. Nutritional information

	Results	Method
Moisture	2.2g/100g	Heat drying method under ordinal pressure
Protein*1	29.2g/100g	Kieldahl method
Fat	0.3g/100g	Acid fat dissolution method
Ash	10.2g/100g	Direct ashing method
Carbohydrate*2	58.1g/100g	
Energy*3	352kcal/100g	
Dietary fiber	0.5g/100g >	Enzymatic-weight method
Sodium	19.8 mg/100g	Atomic absorption spectrophotometory

*1) *N*=6.25

*2) 100 - (moisture + protein + fat + ash + dietary fiber)

*3) Factors for calculating the energy value: protein, 4; fat, 9; available carbohydrate (carbohydrate + dietary fiber), 4

Test trustee: Japan Food Research Center Foundation

Date of issue of the test result report: August 19, 2003

Research result issue number: No. 303080129-001

8. Safety profile

(1) Residual agricultural chemicals

	Results	Detectable limit	Method
BHC	Not detected	0.02ppm	GC
DDT	Not detected	0.02ppm	GC
Aldrin	Not detected	0.01ppm	GC
Dieldrin	Not detected	0.01ppm	GC
Endrin	Not detected	0.01ppm	GC
Diazinon	Not detected	0.05ppm	GC
Parathion	Not detected	0.05ppm	GC
Marathion	Not detected	0.05ppm	GC

Test trustee: Japan Food Research Center Foundation

Date of issue of the test result report: August 19, 2003

Research result issue number: No. 303080129-001

	Results (ppm)	Limit (ppm)	Detectable limit (ppm)
Aldicerb	Not detected	0.10	0.01
Amitorole	Not detected	Not detected	0.02
Bioresmethrin	Not detected	0.1	0.01
Captafol	Not detected	Not detected	0.05
Chlorothalonil	Not detected	0.2	0.02

Cyhexatin	Not detected	Not detected	0.02
Cypermethrin	Not detected	0.05	0.05
Cyproconazole	Not detected	0.1	0.05
Daminozide	Not detected	Not detected	0.10
Deltamethrin	Not detected	2.0	0.05
Dichlorvos	Not detected	0.2	0.01
Fulazifop butyl	Not detected	0.1	0.02
Flucythrinate	Not detected	0.05	0.05
Glyphosate	Not detected	1.0	0.01
Hexaconazole	Not detected	0.05	0.01
Oxamyl	Not detected	0.10	0.01
Permethrin	Not detected	0.05	0.05
Prochloraz	Not detected	0.2	0.05
Propiconazole	Not detected	0.1	0.05
T-2,4,5	Not detected	Not detected	0.05
Triazophos	Not detected	Not detected	0.03

(2) Acute toxicity (LD_{50})

Coffee Bean Extract (1500 mg/kg) was orally administered to fasted, 5-wk old male and female ICR mice for 14 days. No death nor abnormal changes in body weight observed. Autopsy results showed no macroscopic abnormalities in organs. The LD_{50} of COFFEE BEAN EXTRACT by oral administration is deduced to be ≥ 1500 mg/kg.

(3) Chronic Toxicity

Diet containing Coffee Bean Extract (1-2%) was given to male and female rats for 4 weeks. No fatal incidence reported and no changes in behaviour observed. However, weight lost observed in rat consuming coffee bean extract 2%.

(4) Mutagenicity

Mutagenicity test was conducted using highly infective typhoid bacillus (TA 100, TA 1555, TA 98, TA 1537) and *E.coli* (WP2uvra). At concentration 1.2-5000µg/ml, no increase in mutated strains observed. Hence, Coffee Bean Extract has no mutagenic effect.

(5) Human Studies (4-week continuous consumption)

Coffee Bean Extract 600mg/day (3x recommended daily dose) was given to 5 normal healthy male (average age of 38.5years) subjects for 4 weeks. Blood pressure, ECG, blood and urine profile tests were assessed. No changes / fluctuations observed in blood pressure, ECG, blood and urine profile tests. Lower blood sugar level was reflected in blood profile test and no signs of anemia observed.

(6) Food additives

Coffee Bean Extract is an approved and listed as antioxidant for food additives other than chemically synthetic products in Japan.

(7) Safety profile of caffeine

Being member of the methyxanthine alkaloids, caffeine possess mild stimulatory effect on CNS, increases adrenaline secretion etc. The sedating effect of caffeine is very mild and few users report loss of control of caffeine intake. The LD_{50}* of caffeine (oral) in mice is 220-250 mg/kg body weight. The maximum content of caffeine in a cup of coffee is 100mg, which correspond to 130-150 cups of coffee per day by a normal adult weighed 60 kg. Moreover, caffeine is not listed in the category of addicting stimulants (American Psychiatric Association, 1994) nor intoxicants in the International Classification of Diseases.**

* Dose causing death in 50% of the animals receiving the substance

** Classification of diseases by the World Health Organization (WHO)

9. Recommended daily dose

Recommended daily dose of Coffee Bean Extract-P: 100mg-200mg

10. Applications

Applications		Indications	Examples
Foods	Weight Loss Products / Sport Enhancement Preparations	Supresses fat absorption	Bevarages, hard & softgel capsules, tablets, cookies, chocolate wafers etc.
		Prevents fat accumulation	
		Promotes lipolysis	
		Promotes fat metabolism	
	Health supplement / Prevention of diabetes mellitus	Delays sugar absorption	
		Inhibits gluconeogenesis	
Cosmetics	Slimming aids	Prevents unsightly cellulite appearance	Slimming lotion / cream, shower gel, anti-cellulite cream etc.
		Promotes weight loss	

COFFEE BEAN EXTRACT, which is highly water-soluble, suitable food and cosmetics preparations.

11. Packaging

COFFEE BEAN EXTRACT –P (Powder, food grade)
COFFEE BEAN EXTRACT –PCR (Powder, cosmetic grade)
5kg Interior packaging: aluminum-coated plastic bag
Exterior packaging: cardboard box

12. Storage

Store in cool, dry place. Avoid humidity.

13. Expression

<Food>
COFFEE BEAN EXTRACT –P
Expression: raw coffee bean extract, green coffee bean extract, non-roasted coffee bean extract
INCI name: Coffea Canephora Seed Extract
<Cosmetics>
COFFEE BEAN EXTRACT –PCR
INCI name: COFFEA ROBUSTA SEED EXTRACT

14. Patent in China

COFFEE BEAN EXTRACT has been patented in China as “complex for diet”.

PRODUCT STANDARD

PRODUCT NAME

COFFEE BEAN EXTRACT-P

(FOOD)

This product is extracted with ethanol from coffee beans, the seeds of *Coffea canephora Linne* (Rubiaceae). It guarantee minimum of 24.0 % chlorogenic acid and 45.0 % chlorogenic acids. This powder is water-soluble.

1. Appearance	Yellowish powder with slight aroma.	
2. Chlorogenic Acid	Min. 24.0 %	(HPLC)
3. Chlorogenic Acid related compounds	Min. 45.0 %	(HPLC)
4. Loss on drying	Max. 10.0 %	(Analysis for Hygiene Chemists 1 g, 105°C, 2 h)
5. Purifying		
(ⅰ)Heavy metals (As Pb)	Max. 30 ppm	(Sodium Sulfide Colorimetric Method)
(ⅱ)Arsenic (As AS_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
6. Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
7. Mould and YeastFungal	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
8. Coliforms	Negative	(Analysis for Hygienic Chemists)

9. Composition

Ingregient	Content
Coffee Bean Extract	100 %

PRODUCT STANDARD

PRODUCT NAME

COFFEE BEAN EXTRACT-PCR

(COSMETIC)

This product is extracted with aqueous ethanol from coffee beans, the seeds of *Coffea canephora* (Rubiaceae). It contains minimum of 25.0 % polyphenols. This powder is water-soluble.

1. Appearance	Yellowish powder with slight unique smell.	
2. Polyphenols	Min. 25.0 %	(Folin-Denis method)
3. Loss on drying	Max. 10.0 %	(Analysis for Hygienic Chemist)
5. Purifying		
(ⅰ)Heavy metals (as Pb)	Max. 30 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(ⅱ)Arsenic (As As_2O_3)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
6. Standard Plate Counts	Max. 1 x 10^2 cfu/g	(Analysis for Hygienic Chemists)
7. Moulds and Yeast	Max. 1 x 10^2 cfu/g	(Analysis for Hygienic Chemists)
8. Coliforms	Negative	(Analysis for Hygienic Chemists)

9. Composition

Ingregient	Contents
Coffea Robusta Seed Extract	100 %

ORYZA OIL & FAT CHEMICAL CO., LTD. striving to develop new functional health ingredient for general health & well-being.

■ **From product planning to OEM** - For any additional information or assistance, please contact :

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Revised points:
Added the data “The effects of Coffee Bean Extract on body weight and fat synthesis and metabolism in rats fed with cholesterol diet (*in vivo*)” in p. 18.

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ORYZA OIL & FAT CHEMICAL CO., LTD.