ORYZA OIL & FAT CHEMICAL CO., LTD.

# *VINE TEA EXTRACT*

## Anti-obesity

**（Suppress fat absorption・promote fat metabolism and decomposition）**

## Hepaprotective

**（Non-alcohol and alcohol induced liver disease）**

## Food material

■ **Vine Tea Extract-P**

**（Powder Food Grade）**

**ORYZA OIL & FAT CHEMICAL CO., LTD.**

**Ver. 1.0 WJB**

# List

# Anti-obestiy · Hepaprotective

# *VINE TEA EXTRACT*

## 1. Introduction

***Ampelopsis grossedentata*** **(AG)**, an ancient, famous and health benefitting plant of grape family, which is also called “Vine Tea”, mainly distributed and used in the southwest of China for thousands of years. AG is a rare, valuable, wild plant used for food and traditional medicine. Usually, AG grows in a natural area with thick fog, and the altitude is in 400~1300 meters (Figs. 1, 2). It had always been reported that AG leaves extract and its main component, ampelopsin has a lot of health benefiting effects, such as, anti-oxidant, anti-diabetic, anti-inflammatory, lowering blood pressure and cholesterol levels, protecting the liver and so on. It is known that AG is rich in flavonoids, amino acids, rare elements and other nutrients. The dry leaves of AG would have white flavonoid crystals on the surface. In hot water, it tastes bitter first and then sweet, which could stimulate saliva secretion and assuage thirst. In addition, in southwest of China, especially in Guizhou of China, people had been drunk it for more than 600 years (Fig. 3). It is said that people took it to prevent and treat high blood pressure, heart disease, cerebrovascular disease, swelling of the throat, constipation, etc. in that era without a doctor [1].

**Fig. 1** Fresh leaves of AG

**Fig. 2** Dry leaves of AG

**Fig. 3** AG tea

In recent years, our living environments have improved annually, while obesity, which is becoming a public health issue in advanced nation. Obesity, usually is defined with not only heavy weight, but also excessive accumulation of body fat (Fig. 4). Usually, obesity is caused by high calorie diet, alcoholism or metabolic disorders, which continues to cause other diseases, such as fatty liver, hypertension, hyperlipidemia, impaired glucose tolerance, chronic kidney disease, cardiovascular disease, etc. It has been estimated that the number of obese adults in worldwide has already tripled since 1975 counted by the World Health Organization (WHO). Seriously, more and more children are becoming overweight and getting a tendency of obesity [2]. Generally, people who suffered from obesity might have liver diseases. In clinical stage of liver damage, the spectrum ranges from the simple steatosis to steatohepatitis, hepatic inflammation and fibrosis, finally causes cirrhosis, cancer, and liver failure. Obese people, at early stage, triglyceride (TG) and cholesterol accumulation are prevalent because of lipid excessive intake and abnormal lipid metabolism. Toward progress of symptom, the treatment strategies of obesity are focus on reducing the lipid absorption and promoting lipid decomposition, also lifestyle modification and exercise are considered to be important for people with obesity. Now, there are only few drugs for treatment of obesity, unfortunately, these medicines generally possess some side effects, for example, inhibition of the appetite, disgust, headache, liver injury and so on. Thus, recently, many dietary supplements have been developed against obesity, especially for weight loss and inhibition of lipid absorption. These dietary supplements are well acquired because of their food properties and lower side effects. For these reasons, lots of institutes and researchers have sought to find the new components and supplements, which are expected to have good effects and lower side effects to against obesity [2, 3].

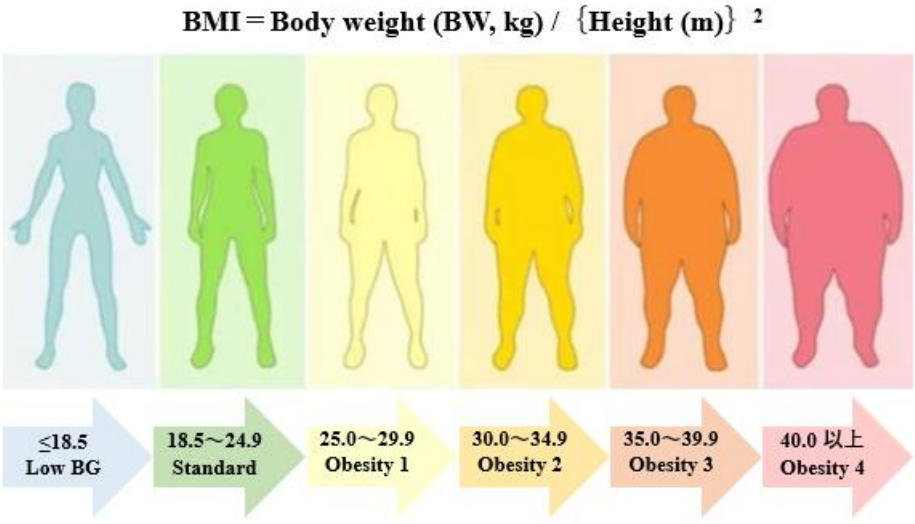


**Fig. 4** Degree of obesity

On the other hand, in 2020, a study by Xie et al. reported that the vine tea extract which contained 65% ampelopsin could prevent western diet-induced NAFLD by

balancing fatty acids oxidation and lipogenesis through increasing the expression of carnitine palmitoyl transferase (CPT) 1A and cytochrome P450, family 4, subfamily a1 (CYP4A1) [4]. According to the records and reports, we speculate that AG extract might have a nice effect against obesity, so here we establish *in vivo* non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) mouse model and *in vitro* 3T3-L1 cell culture system to verify our hypothesis. Meanwhile, the potential mechanisms of AG extract and ampelopsin were summarized and discussed.

**References:**

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# 2. Effective ingredients of Vine Tea

## 2.1 Effective ingredients of vine tea

Leaves of vine tea contain about 35% flavonoids, and its main component is ampelopsin (Fig.5), besides this, it contains lots of other components, such as methionine, amino acids, proteins, fats, carotene, and vitamin E, selenium, iron, zinc, calcium, copper, magnesium, etc.

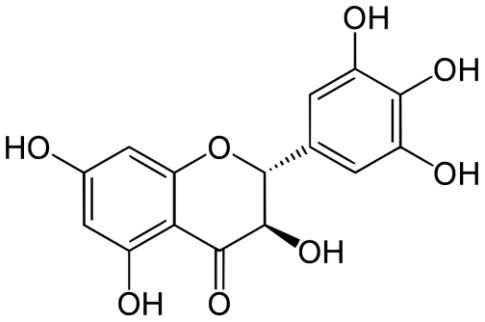



**Fig. 5** Structure of ampelopsin

## 2.2 HPLC determination of ampelopsin and caffeine

Compared to the other teas, no caffeine is a major feature of vine tea (Fig. 6-8).

The chromatograph of AG extract (50% ampelopsin) is showed in Fig. 6, the chromatograph of 98% caffeine is showed in Fig. 7, the chromatograph of a mixture of AG extract and caffeine is showed in Fig. 8. From the graphs, although the retention time of these two components is relatively close, but it was clarified because there was a difference in the retention time between ampelopsin and caffeine, and we could confirm that caffeine was not contained in the vine tea extract.

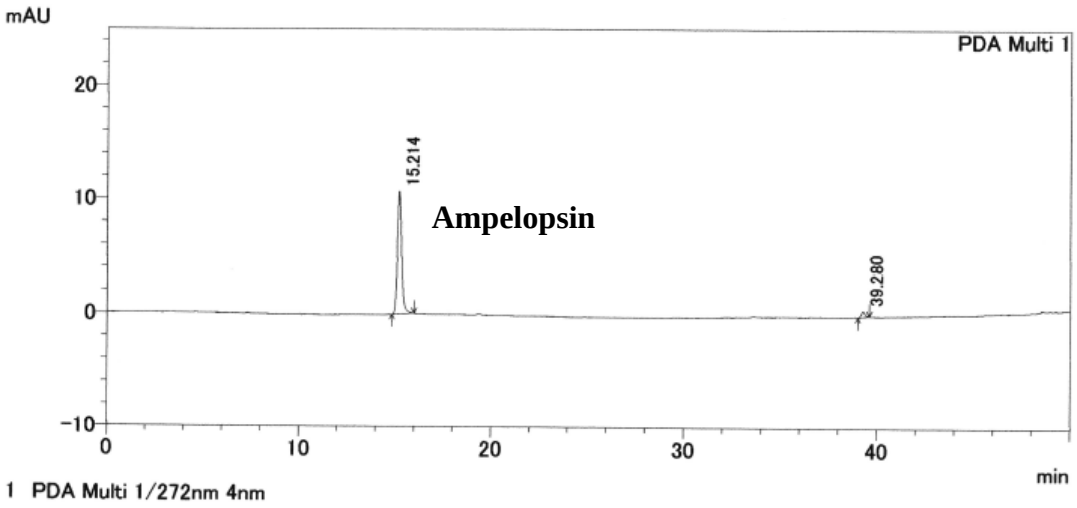



**Fig. 6** The chromatograph of AG extract (50% ampelopsin)

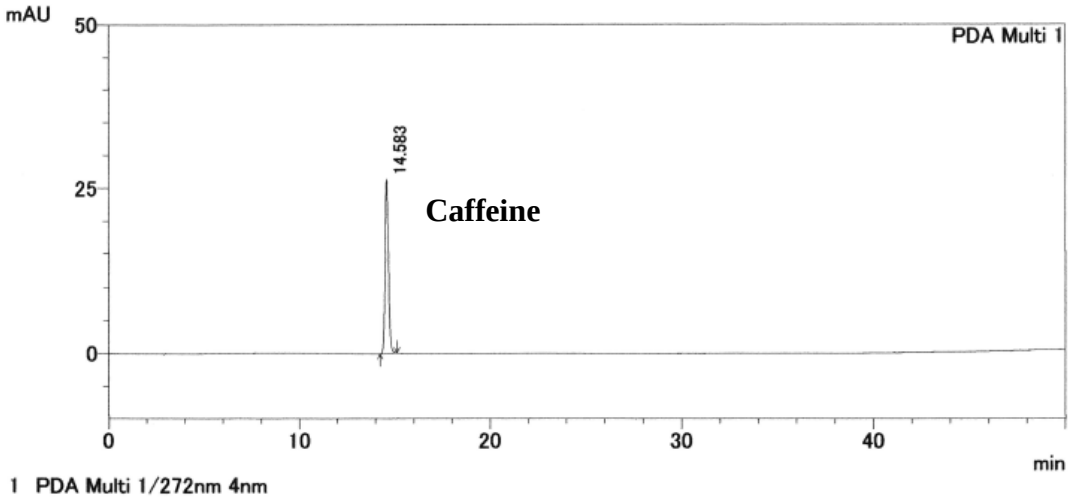



**Fig. 7** The chromatograph of caffeine

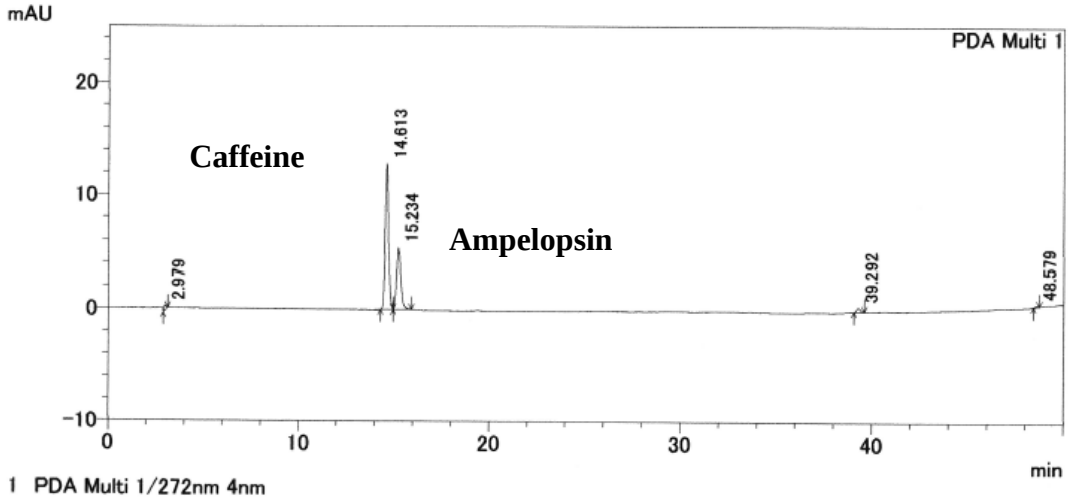



**Fig. 8** The chromatograph of AG extract (50% ampelopsin) and caffeine

# 3. Effects of anti-obesity

## 3.1 *In vivo* experiments

### 3.1.1 Suppress fat absorption-serum TG changes in olive oil-loaded mice

Mice (ICR aged 6 weeks) were fasted for 15 hours and blood samples were collected from orbital sinus under anesthesia by a glass capillary. Thirty minutes later, AG extract (250, 500 mg/kg), ampelopsin (250 mg/kg) and orlistat (20 mg/kg) were given orally to the mice. Then one hour later, olive oil (5 mL/kg) was orally loaded and blood samples were collected at 2, 4 and 6 hours later. The blood samples were centrifuged (3,000 rpm, 10 min) to obtain the serum. TG were measured by Triglyceride E test Wako kit [1].

As showed in Table 1 and Fig. 9, serum TG in control group was elevated compared with normal non-loaded mice, also a reduction effect was observed in AG extract and ampelopsin treated groups. In AG extract (250, 500 mg/kg) treated groups, a significantly decrease in serum TG was observed at 2 and 4h, in ampelopsin (250 mg/kg) treated groups, a significantly decrease in serum TG was observed at 2h. On the other hand, orlistat (20 mg/kg) also showed a significantly inhibitory effect on the increase of serum TG at 2, 4 and 6h.

**Table 1** Effects of AG extract and ampelopsin on lipid absorption in olive oil-loaded mice

| | **Dose (mg/kg)** | **Serum TG（mg/dL）** | | | |
|---|---|---|---|---|---|
| | | 0h | 2h | 4h | 6h |
| **Normal** | - | 93.2±5.5 | 123.8±14.1 | 142.7±10.8 | 122.9±19.0 |
| **Control** | - | 138.4±16.5 | 812.1±68.9 | 564.2±33.0 | 303.5±27.7 |
| **AG extract** | **250** | 146.5±16.4 | 530.7±36.5** | 399.8±45.6* | 222.8±33.9 |
| **AG extract** | **500** | 154.9±18.3 | 571.7±53.2* | 431.4±34.5* | 210.6±21.6* |
| **Ampelopsin** | **250** | 145.8±23.2 | 588.7±55.0* | 452.5±18.9 | 231.4±36.0 |
| **Orlistat** | **20** | 113.5±10.7 | 387.1±79.2** | 249.2±58.5** | 140.3±24.0** |

Each symbol represents the mean with SE (n=6). Asterisks denote significant differences from the control group at *$p < 0.05$, ** $p<0.01$.

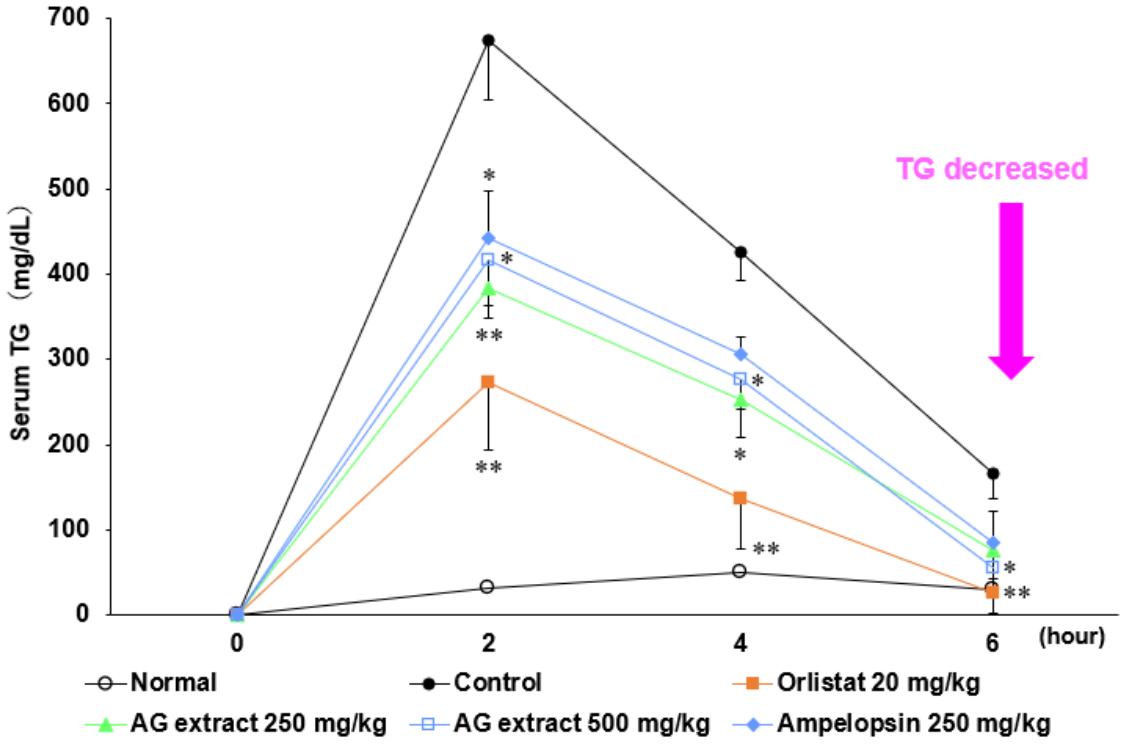



**Fig. 9** Effects of AG extract and ampelopsin on lipid absorption in olive oil-loaded mice.

Each symbol represents the mean with SE (n=6). Asterisks denote significant differences from the control group at *$p < 0.05$, ** $p<0.01$.

Using the same test method as 3.1.1, we compared the effects of fat absorption inhibitory between vine tea extract and Kudzu flower extract (sample produced by Oryza). As showed in Table 2 and Fig. 10, serum TG in control group was elevated compared with normal non-loaded mice. While in AG extract (250 mg/kg) treated group and Kudzu flower extract (containing 28% tectorigenin, 250 mg/kg), a significant decrease in serum TG was observed at 6h. In addition, in AG extract (250 mg/kg) treated group, a significant decrease in serum TG was observed at 4h.

**Table.2** Effects of fat absorption inhibitory between vine tea extract and Kudzu flower extract

| | Dose (mg/kg) | Serum TG（mg/dL） | | | |
|---|---|---|---|---|---|
| | | 0h | 2h | 4h | 6h |
| **Normal** | - | 219.0±54.5 | 358.5±82.1 | 322.0±46.3 | 254.1±36.7 |
| **Control** | - | 154.3±34.9 | 880.3±137.1 | 1096.8±233.6 | 593.5±126.1 |
| **AG extract** | **250** | 114.4±26.2 | 524.7±84.4 | 472.1±101.2* | 212.1±80.1* |
| **Kudzu flower extract** | **250** | 92.6±22.5 | 711.0±87.0 | 770.8±97.6 | 302.7±63.1* |

Each symbol represents the mean with SE (n=4-6). Asterisks denote significant differences from the control group at *$p < 0.05$.

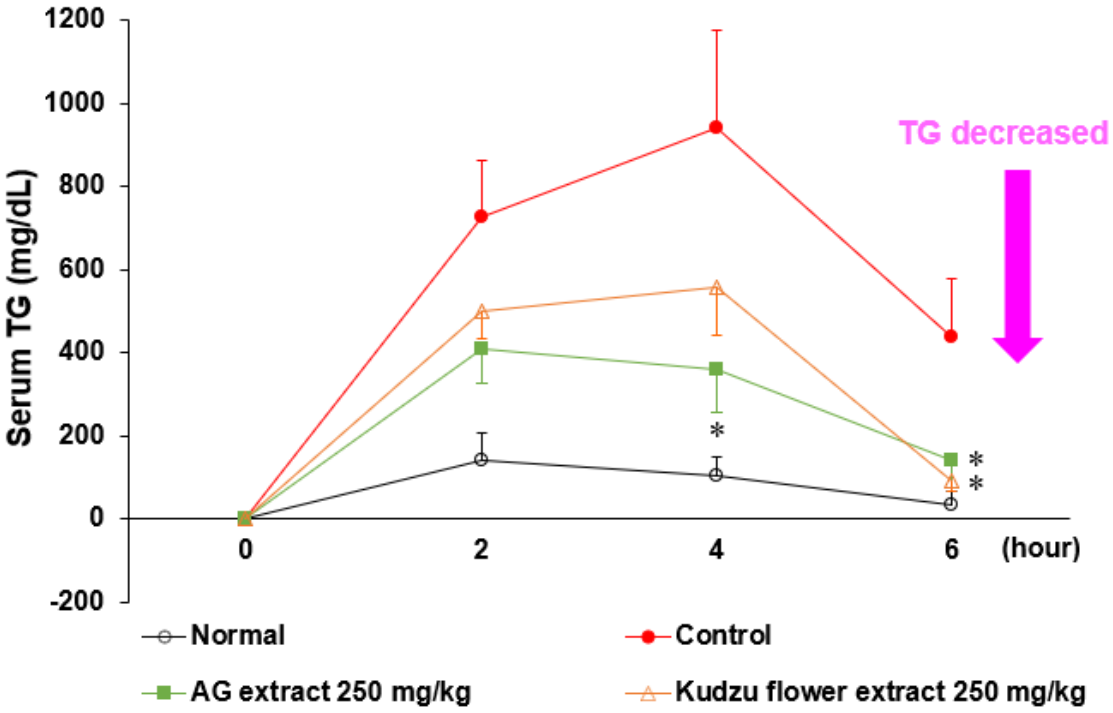



**Fig. 10** Effects of fat absorption inhibitory between vine tea extract and Kudzu flower extract

Each symbol represents the mean with SE (n=4-6). Asterisks denote significant differences from the control group at *$p < 0.05$.

### 3.1.2 Suppress fat accumulation in high fat diet (HFD)-fed mice

#### 3.1.2.1 Effects of AG extract and ampelopsin on body weight in HFD-fed mice

In high fat diet-fed mice, compared to the control group, from day 6, the body weight of AG extract (250, 500 mg/kg) and ampelopsin (250, 500 mg/kg) treated groups were suppressed (Fig. 11).

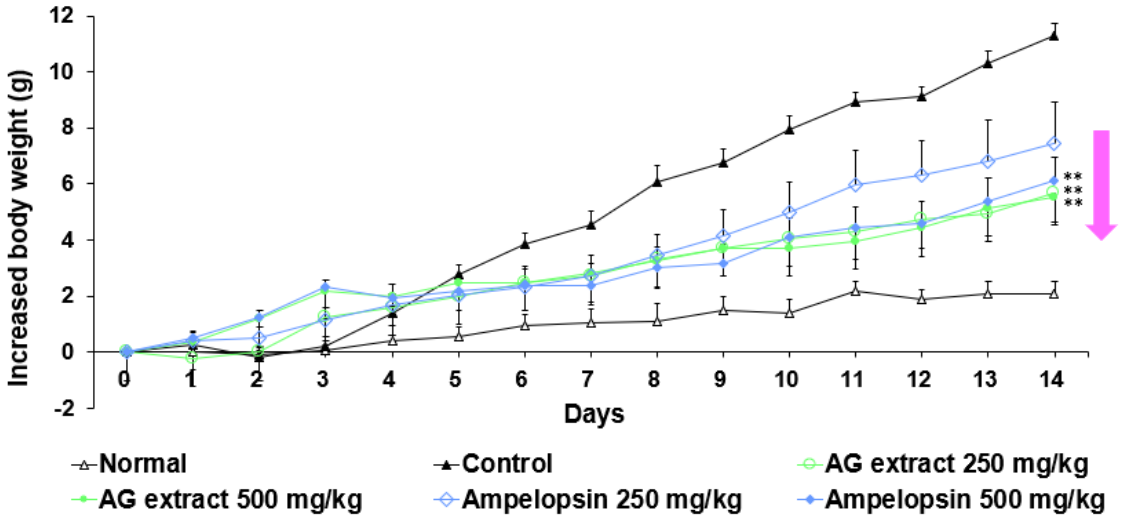
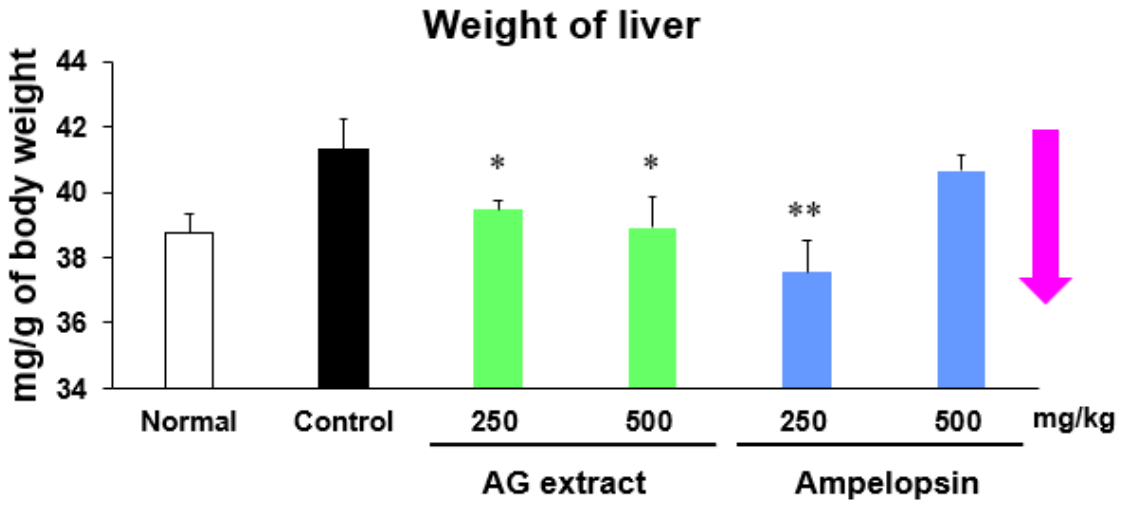



**Fig. 11** Effects of AG extract and ampelopsin on HFD-induced body weight change in mice.

Each symbol represents the mean with SE (n=6-7). Asterisks denote significant differences from the control group at ** $p<0.01$.

#### 3.1.2.2 Effects of AG extract and ampelopsin on weight of liver, epididymal fat and perirenal fat in HFD-fed mice

Compared to the control group, on day 14, the weight of liver, epididymal fats, perirenal fats of AG extract and ampelopsin treated groups were also suppressed (Fig. 12).



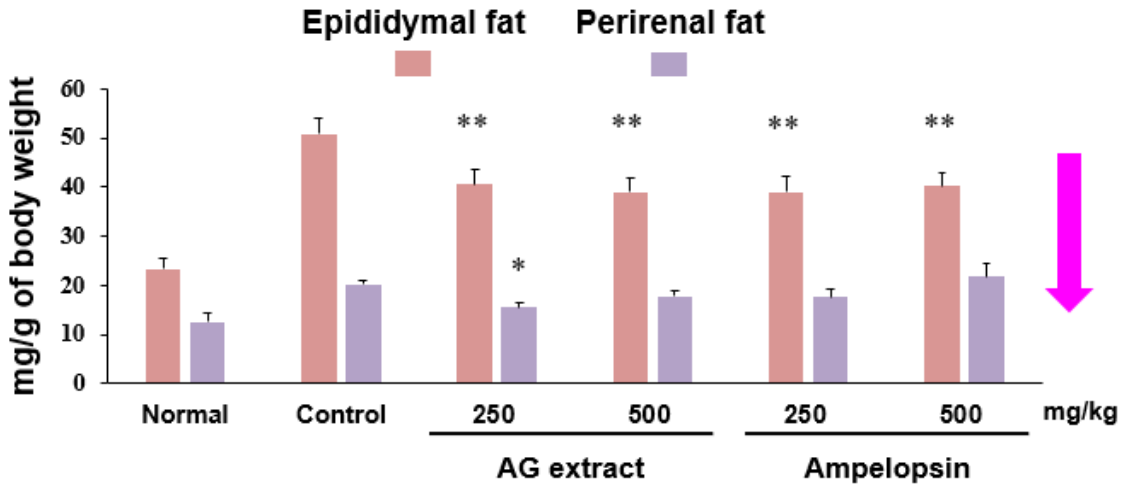



**Fig. 12** Effects of AG extract and ampelopsin on body weight, and weight of liver, epididymal fat and perirenal fat in HFD-fed

Each value represents the mean with SE (n=6-7). Asterisks denote significant differences from the control group at *p<0.05, ** p<0.01.

### 3.1.2.3 Effects of AG extract and ampelopsin on serum TG in HFD-fed mice

Compared to the control group, serum TG of AG extract and ampelopsin treated groups were also reduced (Fig. 13), especially in AG extract (250 mg/kg) and ampelopsin (250 mg/kg) group.

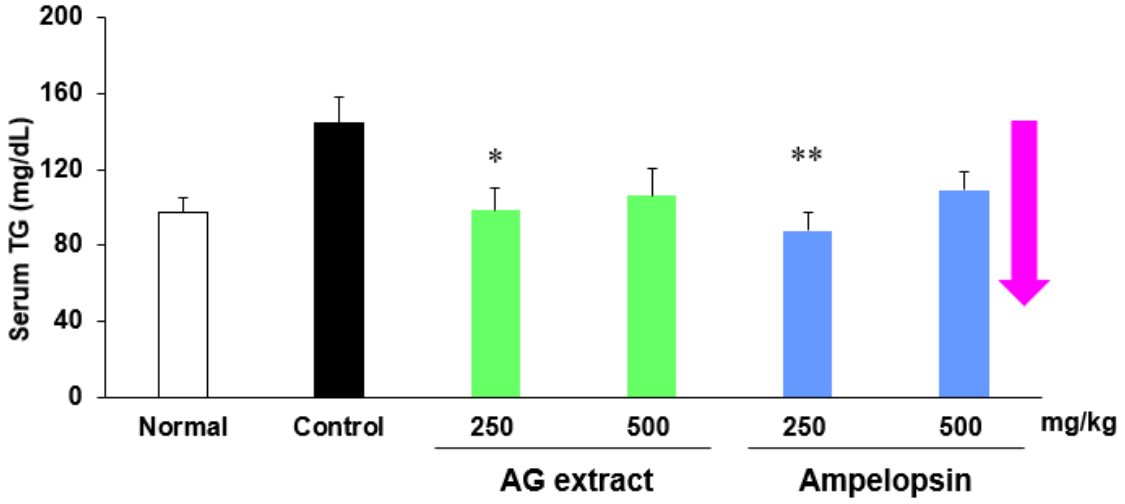



**Fig. 13** Effects of AG extract and ampelopsin on serum TG in HFD-fed mice

Each value represents the mean with SE (n=6-7). Asterisks denote significant differences from the control group at *p<0.05, ** p<0.01.

### 3.1.2.4 Mechanism of suppressing fat accumulation in high fat diet (HFD)-fed mice

Free fatty acids decomposed and released from adipose tissue are transported to the liver, and then metabolized by mitochondria of hepatocytes, this process is called

β-oxidation. CPT is considered as a key enzyme in the carnitine-dependent transport across the mitochondrial inner membrane in the β-oxidation of long-chain fatty acids. In addition, it has been reported that vine tea extract could increase CPT1A activity in liver [2]. In this study, we also examined the expression of CPT1A in liver of HFD fed-mice, the results showed that compared to the control group, an increasing effect on CPT1A was observed in AG extract and ampelopsin group (Fig. 14, 15). From this result, it is considered that AG extract and ampelopsin possess the effect of promoting fat burning.

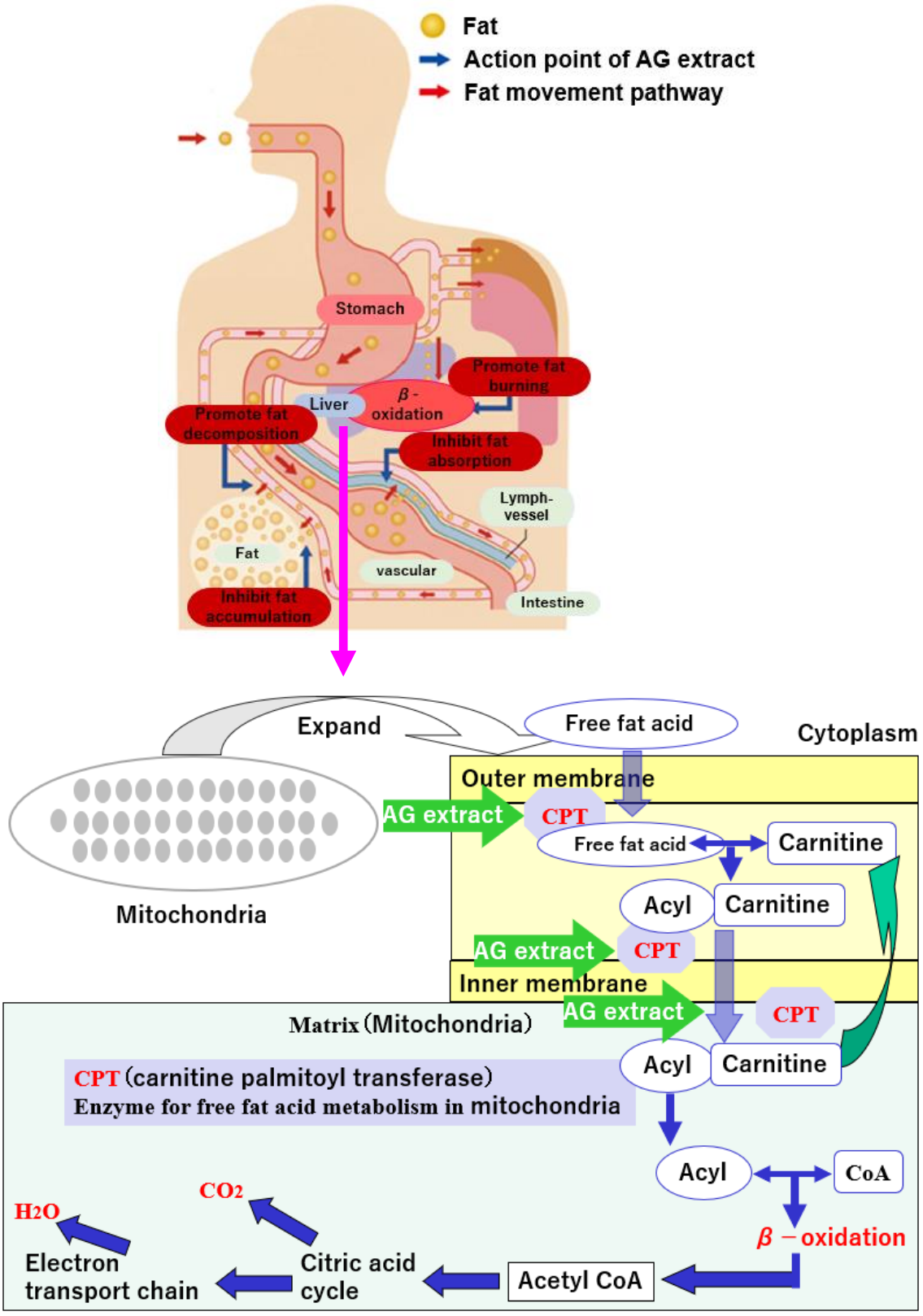



**Fig. 14**. Metabolism of AG extract in liver

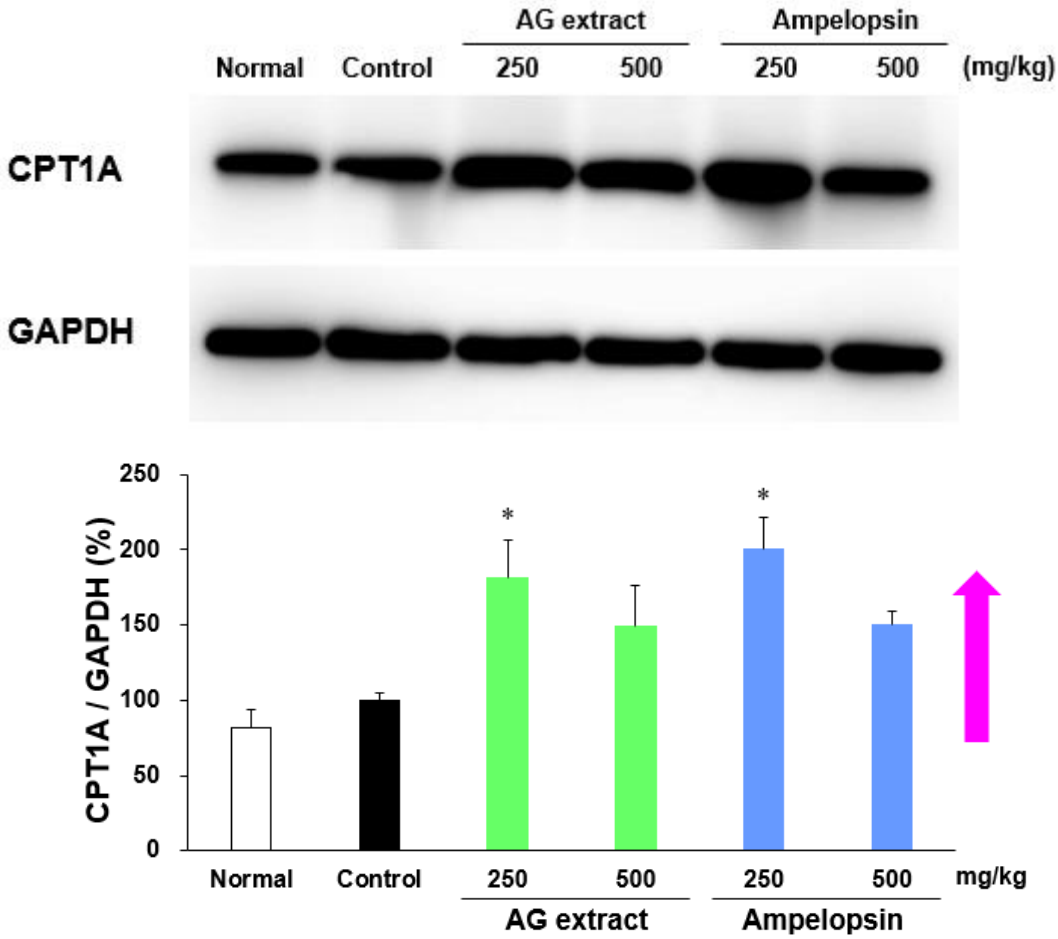



**Fig. 15** Effects of AG extract and ampelopsin on CPT1A protein expression in the liver of HFD-fed mice.

Each column represents the mean with SE (n=3-4), Asterisks denote significant differences from the control group at $*p < 0.05$.

## 3.2 *In vitro* experiments

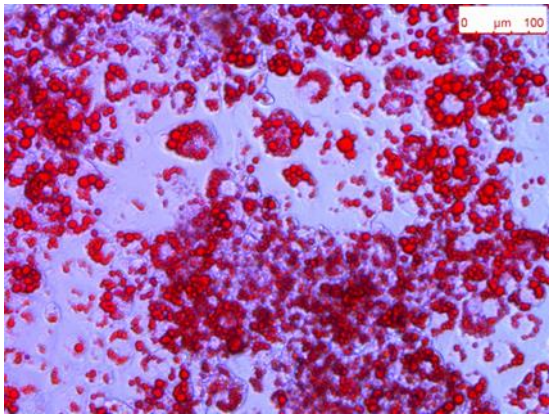
### 3.2.1 Promoting fat decomposition (3T3-L1 cell line)

3T3-L1 cells, in the medium with insulin, would be differentiated from pre-adipocytes into adipocytes (morphological change), which continued to cause TG accumulation. AG extract and ampelopsin suppressed this process in reducing TG accumulation, cell size and lipid droplets (Table 3, Fig. 16) [3]. Compared to the control group, the Oil Red O staining showed that AG extract significantly decreased TG accumulation in 3T3-L1 adipocytes (The absorbance and lipid droplets were reduced).

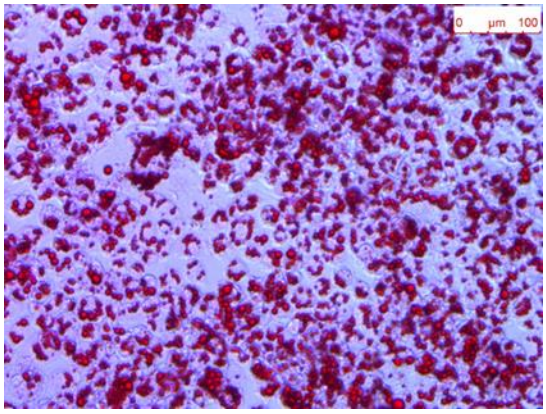
**Table 3** TG contents of 3T3-L1 cells treated with AG extract and ampelopsin.

| | | Concentration (μg/mL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | 1 | 3 | 10 | 30 | 100 |
| TG (%) | AG extract | 100.0±1.1 | 90.1±2.5 | 89.9±2.2** | 85.6±1.2** | 92.7±2.1* | 85.2±1.2** |
| | Ampelopsin | 100.0±3.2 | 99.7±2.1 | 90.0±4.2 | 99.2±6.0 | 91.7±4.3 | 83.2±1.7* |

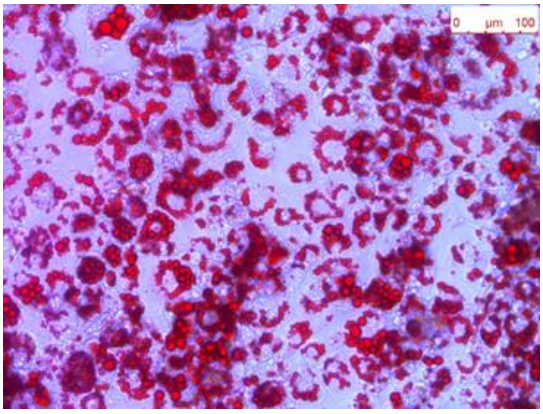
Each TG value represents the mean with SE (n=3-4). Asterisks denote significant differences from the control group at *$p$<0.05, ** $p$<0.01.




Control




AG extract 30 μg/mL




Ampelopsin 30 μg/mL

**Fig. 16** Effects of AG extract and ampelopsin on adipogenesis in differentiated 3T3-L1 adipocytes.

### 3.2.2 Promoting fat decomposition (rat fat pad)

Compared to the control group, the contents of free fatty acid (FFA) and glycerol released from the rat epididymal fat pad were significantly increased in AG extract and ampelopsin groups [4-5], which suggested that AG extract and ampelopsin could promote fat decomposition (Fig. 17).

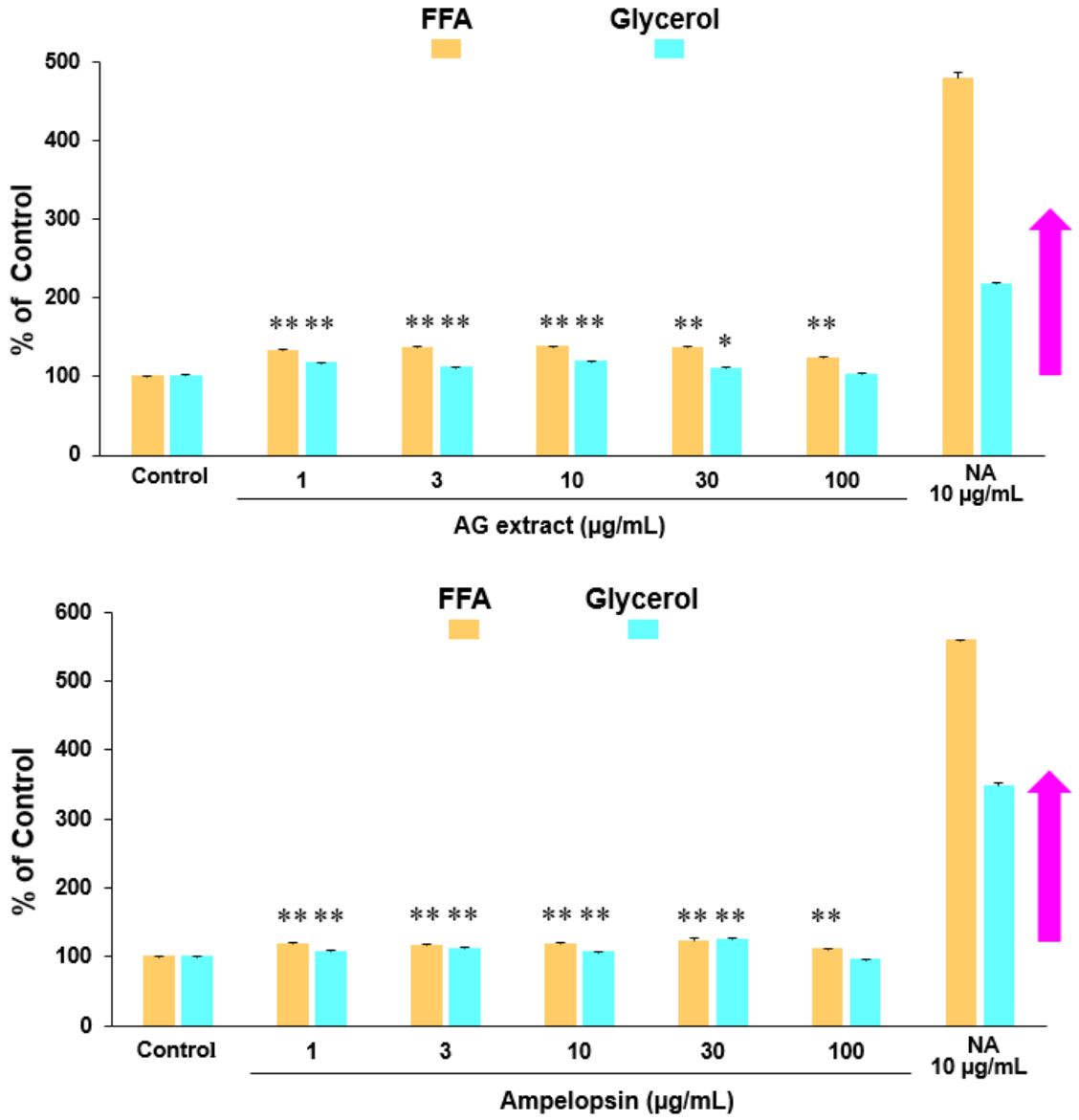



**Fig. 17** Lipolytic effects of AG extract and ampelopsin on rat epididymal fat pad.
Each column represents the mean with SE (n=3). Asterisks denote significant differences from the control group at *$p < 0.05$, ** $p<0.01$.

### 3.2.3 Effects of AG extract and ampelopsin on pancreatic lipase activity

From mice experiments, we had known that AG extract and ampelopsin could suppress fat absorption. Usually, *in vivo*, fat absorption is connected with pancreatic lipase activity, so here we evaluated the effects of AG extract and ampelopsin on pancreatic lipase activity *in vitro*. As a result, compared to the control group, AG extract and ampelopsin inhibited the pancreatic lipase activity significantly (Fig. 18).

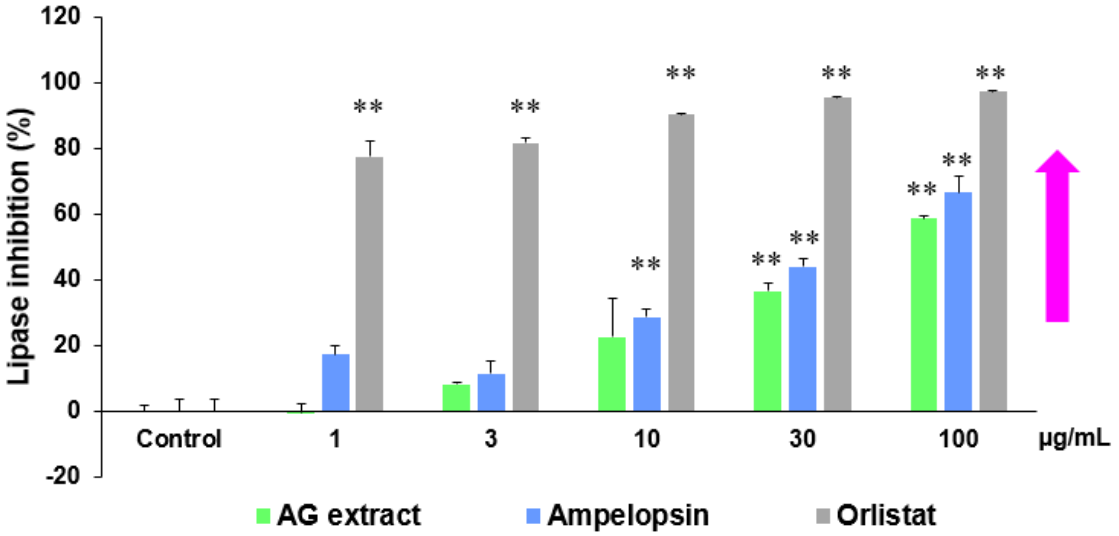



**Fig. 18** Pancreatic lipase inhibition of AG extract and ampelopsin.
Each value represents the mean with the SE (n=3). Asterisks denote significant differences from the control group at ** $p$<0.01.

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## 3.3 Clinical trials：

### 3.3.1 Anti-obesity

A double-blind comparative study of the effects of Vine Tea extract-P (containing 50% ampelopsin) 250 mg/day or placebo for 3 consecutive months in 27 healthy males and females aged 25 to 65 years were engaged. As a result, compared to initial, the visceral fat area, abdominal circumference, body weight, and BMI were reduced in Vine Tea extract-P intake group. Compared to placebo group, the visceral fat area was decreased significantly after taking Vine Tea extract-P for 1 month, also a continuous decreasing tendency was observed after 2 months and 3 months intake. Therefore, Vine Tea extract-P could be expected to have good anti-obestiy effect in reducing the visceral fat area.

**Table 4** Detailed data after taking Vine Tea extract-P for 3 months in human

| Items | Unit | | 0 month | 1 month | 2 months | 3 months | Variation | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 1 month | 2 months | 3 months |
| Visceral fat area | $cm^2$ | Placebo | 79.2±40.1 | 77.8±38.4 | 74.6±37.5 | 69.4±37.3 | -1.4±6.5 | -4.6±9.3 | -9.8±9.7 |
| | | Vine Tea extract-P | 106.7±63.8 | 96.9±62.5* | 94.6±66.3* | 92.4±61.9* | **-9.8**±12.5† | **-12.0**±17.6 | **-14.3**±19.7 |
| Abdominal circumference | cm | Placebo | 81.9±8.4 | 82.0±8.3 | 80.6±7.4* | 80.3±7.5* | 0.1±1.1 | -1.3±1.8 | -1.5±2.2 |
| | | Vine Tea extract-P | 88.5±11.6 | 88.7±11.6 | 86.9±12.4 | 86.7±11.5* | 0.2±2.0 | **-1.7**±3.0 | **-1.8**±2.6 |
| Body weight | kg | Placebo | 62.6±8.7 | 62.2±8.5 | 61.8±8.3* | 61.6±8.4 | -0.4±0.8 | -0.8±1.0 | -0.9±1.5 |
| | | Vine Tea extract-P | 70.9±11.3 | 70.9±11.1 | 70.1±11.6* | 70.2±11.4 | 0.1±0.8 | -0.7±1.2 | -0.7±1.5 |
| Body fat mass | kg | Placebo | 15.8±4.2 | 15.4±4.1 | 15.2±4.0 | 14.8±3.7* | -0.4±1. | -0.6±1.0 | -1.0±1.1 |
| | | Vine Tea extract-P | 20.0±7.7 | 20.2±7.6 | 20.0±7.9 | 19.6±7.5 | 0.2±0.5 | 0.0±0.9 | -0.3±1.3 |
| BMI | $kg/m^2$ | Placebo | 22.7±2.2 | 22.6±2.2 | 22.4±2.2* | 22.4±2.1 | -0.1±0.3 | -0.3±0.4 | -0.3±0.5 |
| | | Vine Tea extract-P | 25.2±3.0 | 25.2±2.9 | 24.9±3.2* | 24.9±3.1 | 0.0±0.3 | -0.3±0.4 | -0.3±0.5 |
| Body fat % | % | Placebo | 24.7±4.7 | 24.8±5.4 | 24.6±5.3 | 24.1±4.8 | 0.1±2.5 | -0.1±2.5 | -0.6±2.3 |
| | | Vine Tea extract-P | 27.7±7.3 | 28.0±7.2 | 27.9±7.4 | 27.5±7.2 | 0.3±0.6 | 0.2±0.8 | -0.2±1.5 |

Each value represents the mean with the SD (n=13). *$p$<0.05, **$p$<0.01 VS baseline (paired t-test), †p<0.05 (unpaired t-test).

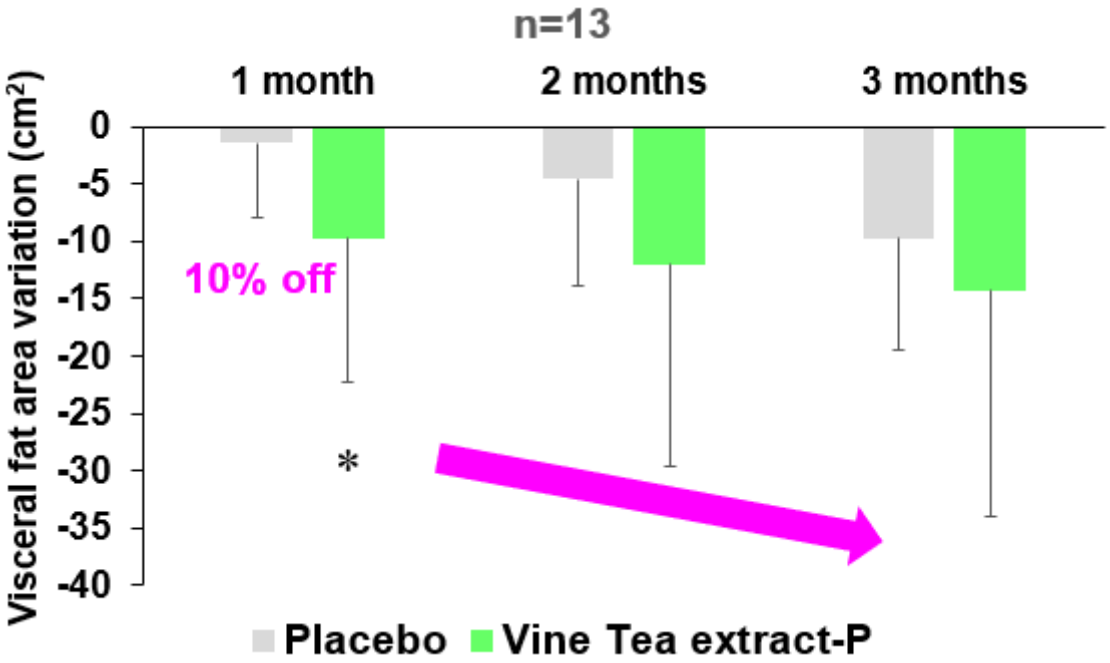



**Fig. 19** Effect of Vine Tea extract-P on visceral fat area of human
(*$p$<0.05 VS baseline)

### 3.3.2 Suppress fat absorption

In order to evaluate the fat absorption inhibitory effect of Vine Tea extract-P (containing 50% ampelopsin), a single-blind crossover study was conducted on healthy males and females (n=10) aged 30 to 61 years. The variation of serum TG concentration (ΔTG) and its curve area AUC (ΔTG AUC) were determined. As a result, after intake of high fat food and test samples for 2h, compared to placebo group, Vine Tea extract-P group suppressed serum TG elevation significantly. In addition, a significantly suppressed effect of ΔTG AUC was also observed in Vine Tea extract-P group. Therefore, it was confirmed that Vine Tea extract-P has good inhibitory effect in fat absorption.

**Table 5** Effect of Vine Tea extract-P on serum TG of human

| Items | | Placebo | AG extract-P |
|---|---|---|---|
| Serum TG AUC (TG AUC、mg・h/dL) | | 297.1±193.1 | 179.9±126.7* |
| Serum ΔTG（ΔTG、mg/dL） | ΔTG（2h-0h） | 109.0±74.0 | 55.1±40.5* |
| | ΔTG（4h-0h） | 79.1±72.6 | 69.7±64.8 |

Each value represents the mean with the SD (n=10). *$p$<0.05, **$p$<0.01 VS placebo group.

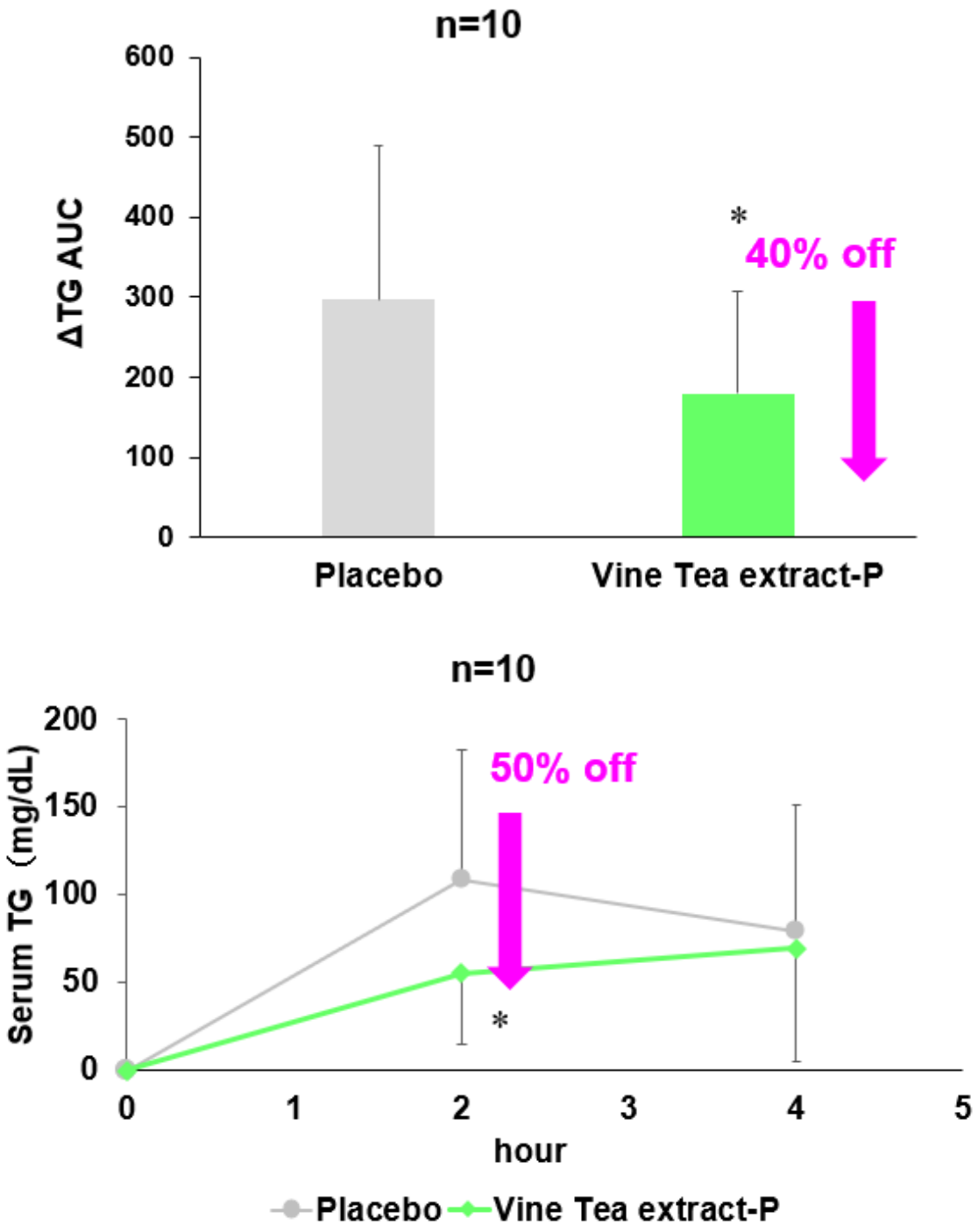



**Fig. 20** Effect of Vine Tea extract-P on serum TG and TG AUC of human

Each value represents the mean with the SD (n=10). *$p$<0.05, **$p$<0.01 VS placebo group.

# 4. Hepaprotective effect

## 4.1 *In vivo* experiments

### 4.1.1 Liver injury test induced by carbon tetrachloride ($CCl_4$)

The model of $CCl_4$ induced liver injury is equivalent to human hepatitis caused by active oxygen and toxic substances, and is considered as a model which could evaluate effect of detoxification [1]. Here, in mice, compared to the control group, AG extract and ampelopsin suppressed the increase of GOT and GPT, which were important parameters of liver function (Fig. 21).

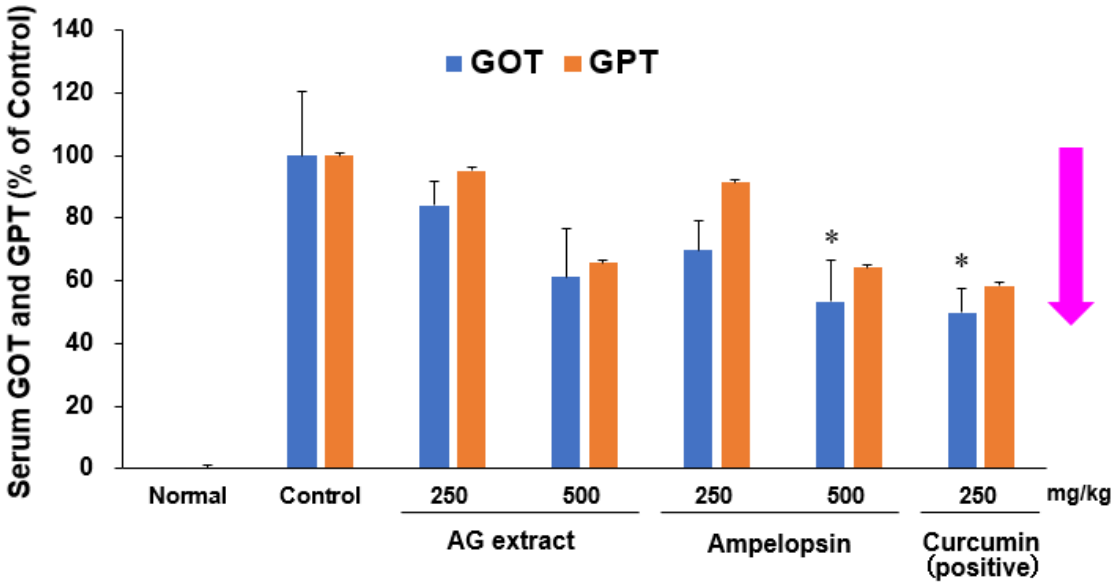



**Fig. 21** Effect of AG extract and ampelopsin on $CCl_4$ induced liver injury in mice
Each value represents the mean with the SE (n=5~6). Asterisks denote significant differences from the control group at ** $p$<0.01.

### 4.1.2 Liver injury test induced by D-Galactosamine and Lipopolysaccharide (D-GaIN+LPS)

The model of (D-GaIN+LPS) induced liver injury is similar to immune response-related liver injury and human viral hepatitis [1]. As a result, compared to the control group, AG extract and ampelopsin were found to suppress the increase of GOT and GPT, which were important parameters of liver function (Fig. 22).

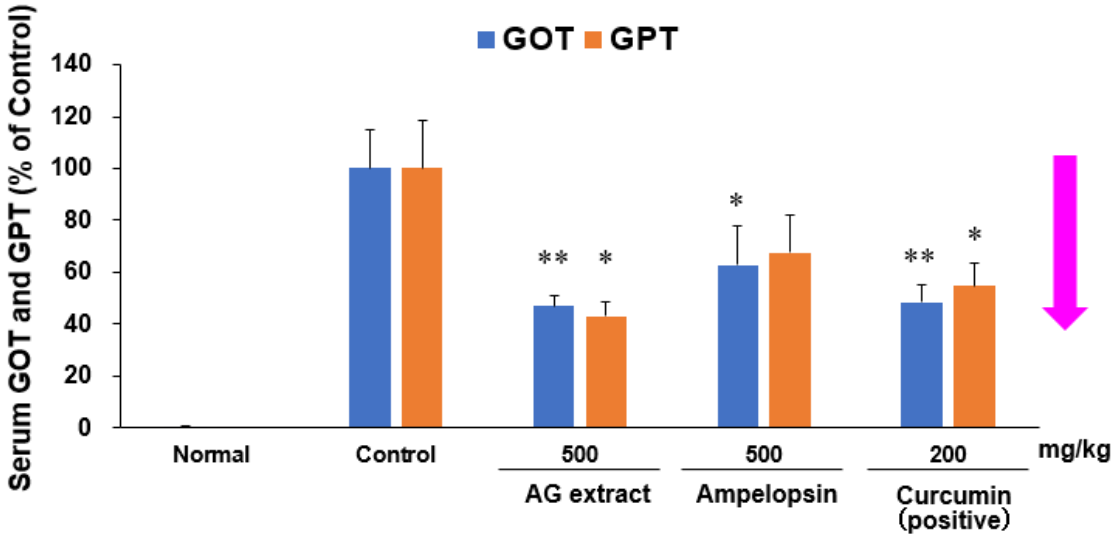



**Fig. 21** Effect of AG extract and ampelopsin on (D-GaIN+LPS) induced liver injury in mice

Each value represents the mean with the SE (n=5~6). Asterisks denote significant differences from the control group at *$p$<0.05, ** $p$<0.01.

### 4.1.3 Alcohol induced liver injury

Alcoholic liver disease is often occurred in people who drink alcohol habitually. People who drink lots of alcohol may cause fat accumulation and inflammation in liver. It had been reported that AG extract and ampelopsin could suppress serum ethanol, GOT, GPT and fat accumulation in mice experiments [2, 3]. According to these reports, we established the alcohol liver disease mice model to confirm the results of AG extract and ampelopsin.

#### 4.1.3.1 Suppress serum alcohol concentration

In alcohol diet-fed mice, compared to the control group, the concentration of serum ethanol was tended to reduce in AG extract, ampelopsin and curcumin treated groups (Fig. 23).

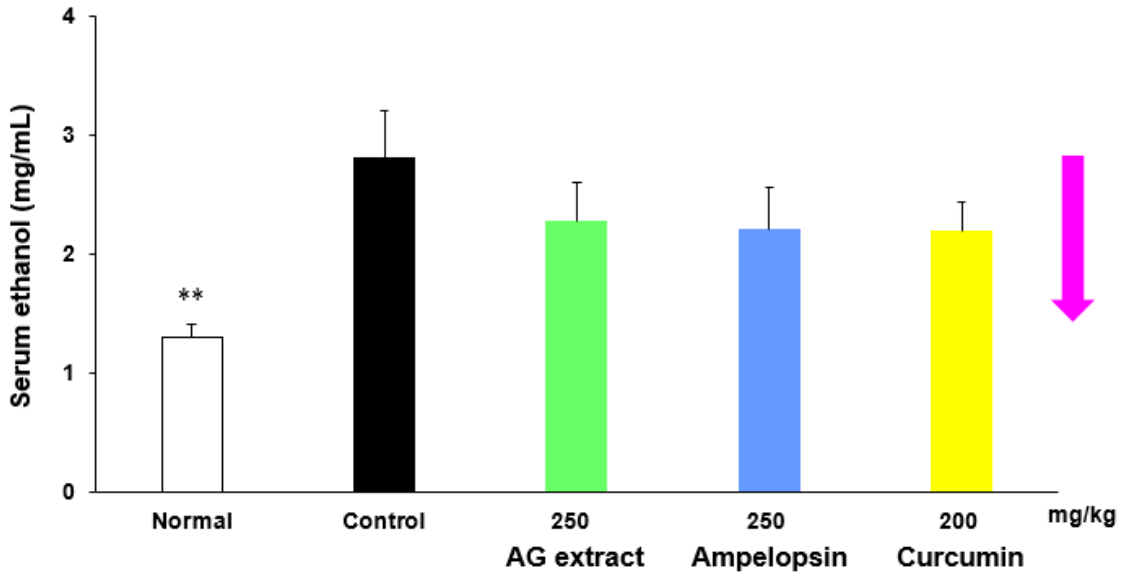



**Fig. 23** Effects of AG extract and ampelopsin on serum ethanol in alcohol diet-fed mice.
Each column represents the mean with SE (n=5-6).

### 4.1.3.2 Suppress serum GOT and GPT

In alcohol diet-fed mice, compared to the control group, the concentration of serum GOT and GPT were tended to reduce in AG extract, ampelopsin and curcumin treated groups (Fig. 24).

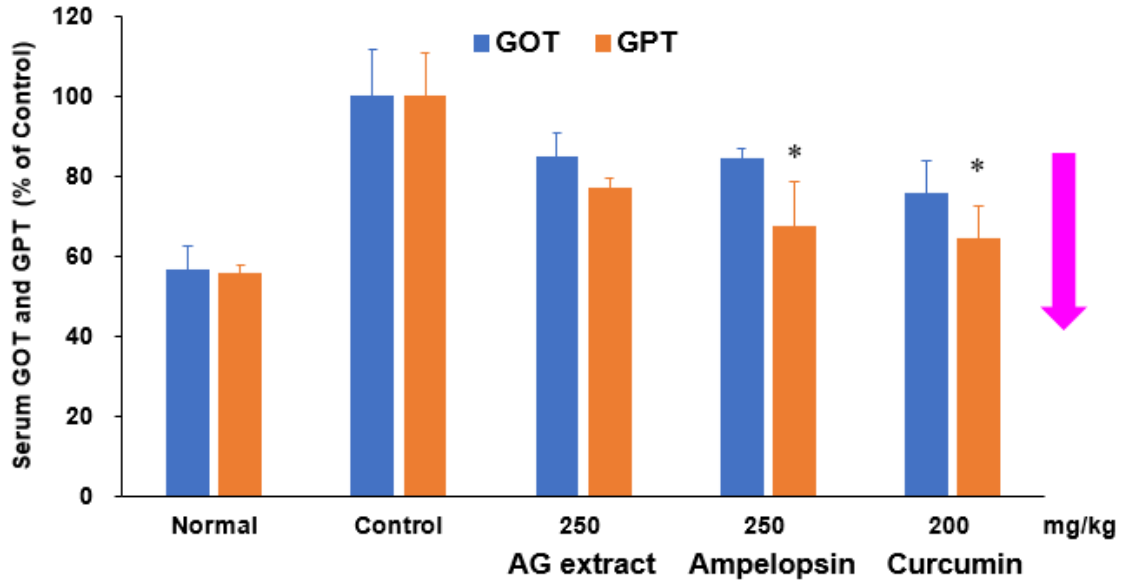



**Fig. 24** Effects of AG extract and ampelopsin on serum GOT and GPT in alcohol diet-fed mice.
Each column represents the mean with SE (n=5-6). Asterisks denote significant differences from the control group at *$p$<0.05.

#### 4.1.3.3 Suppress liver TG and hepatic microscopic image

In alcohol diet-fed mice, compared to the control group, the contents of TG in liver were also significantly reduced in AG extract, ampelopsin and curcumin treated groups (Fig. 25). Meanwhile, in HE-stained liver sections, compared to the control group, an inhibitory effect of TG accumulation in liver was confirmed in AG extract, ampelopsin treated groups (Fig. 26).

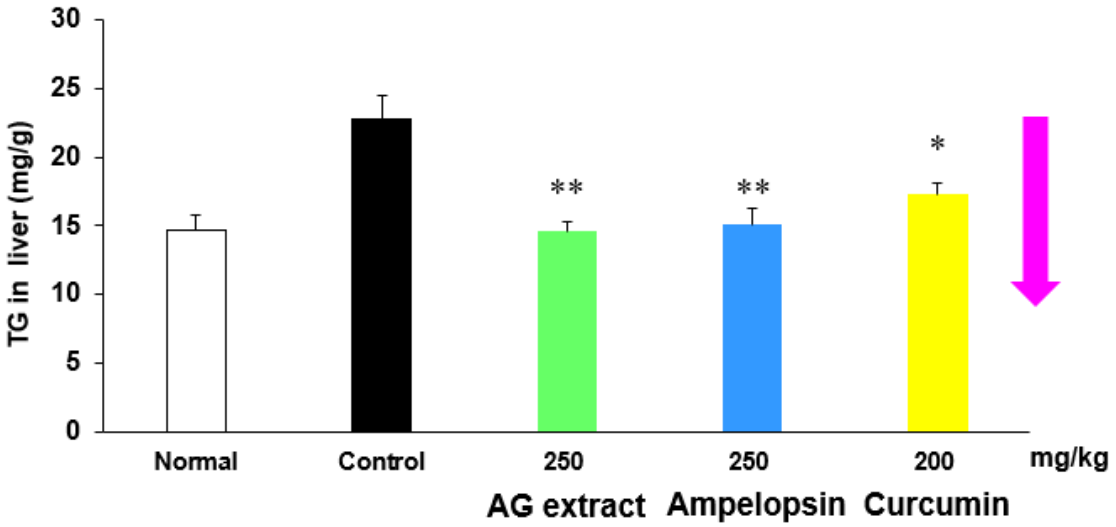



**Fig. 25** Effects of AG extract and ampelopsin on liver TG in alcohol diet-fed mice.
Each column represents the mean with SE (n=5). Asterisks denote significant differences from the control group at *$p$<0.05, **$p$<0.01.

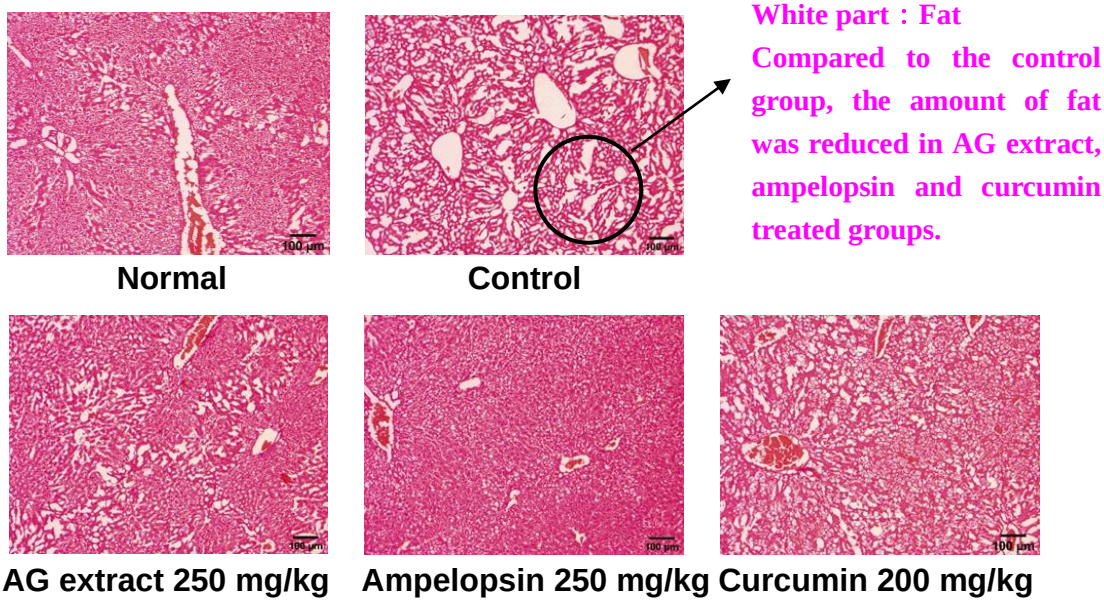



**Fig. 26** Microscopic hematoxylin-eosin staining (HE) images of liver sections.

#### 4.1.3.4 Mechanism of AG extract and ampelopsin on alcohol diet-fed mice

The main pathway for alcohol metabolism involves two enzymes, alcohol dehydrogenase (ADH) and Aldehyde dehydrogenase (ALDH). These two enzymes contribute to the degradation of alcohol and its elimination from the body. In the first step, ADH metabolizes ethanol to acetaldehyde, a highly toxic substance that is a known carcinogen. In the second step, acetaldehyde is metabolized by ALDH to less toxic acetate, which is further broken down into water and carbon dioxide for elimination (Fig. 27). Meanwhile, an excessive alcohol intake may activate the hepatic microsomal ethanol-oxidizing system (MEOS). CYP2E1 is considered to be the most important enzyme in MEOS. When ethanol is metabolized by CYP2E1, highly reactive oxygen-containing molecules or reactive oxygen species are produced. These free radicals deplete intracellular defenses against oxidative stress, further damaging hepatocyte proteins and DNA, or interact with other substances to create carcinogens. Here, in this study, in comparisons with the control group, hepatic ALDH2 was elevated in the groups treated with AG extract (250 mg/kg), ampelopsin (250 mg/kg), and curcumin (200 mg/kg) (Fig. 28), while a significant decrease in hepatic CYP2E1 was observed in the groups treated with AG extract (250 mg/kg), ampelopsin (250 mg/kg), and curcumin (200 mg/kg) (Fig. 28). These results indicated that the potential of AG extract and ampelopsin to protect the liver from alcohol-induced fatty liver more than promoting the metabolism of alcohol.

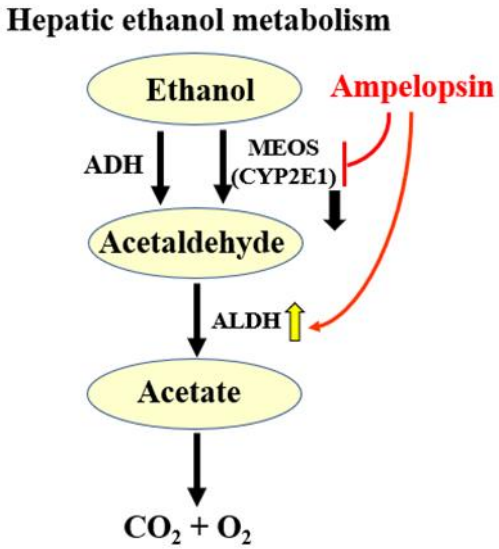



**Fig. 27** Potential underlying pathway by which AG extract and ampelopsin contribute to ethanol metabolism in the liver

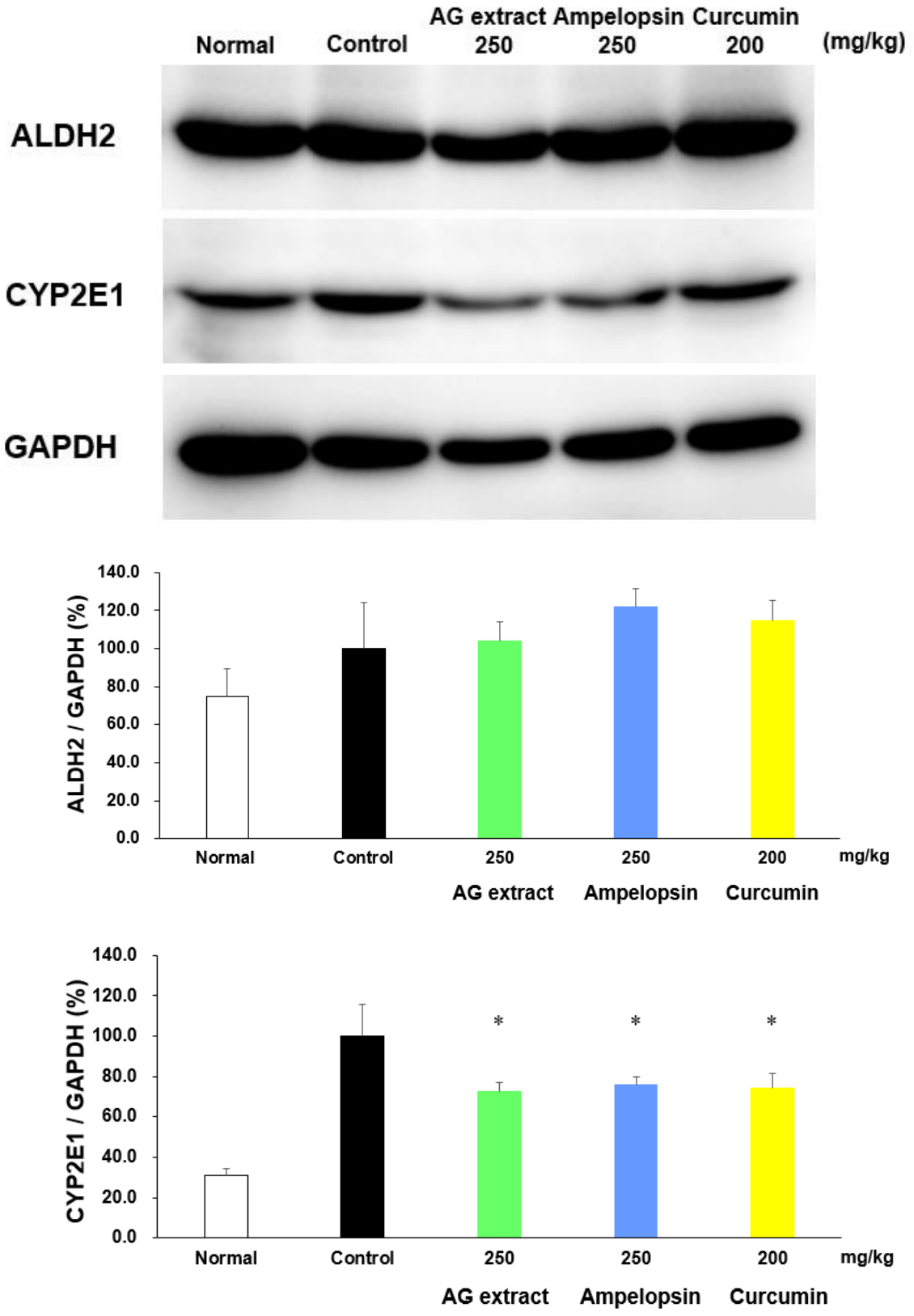
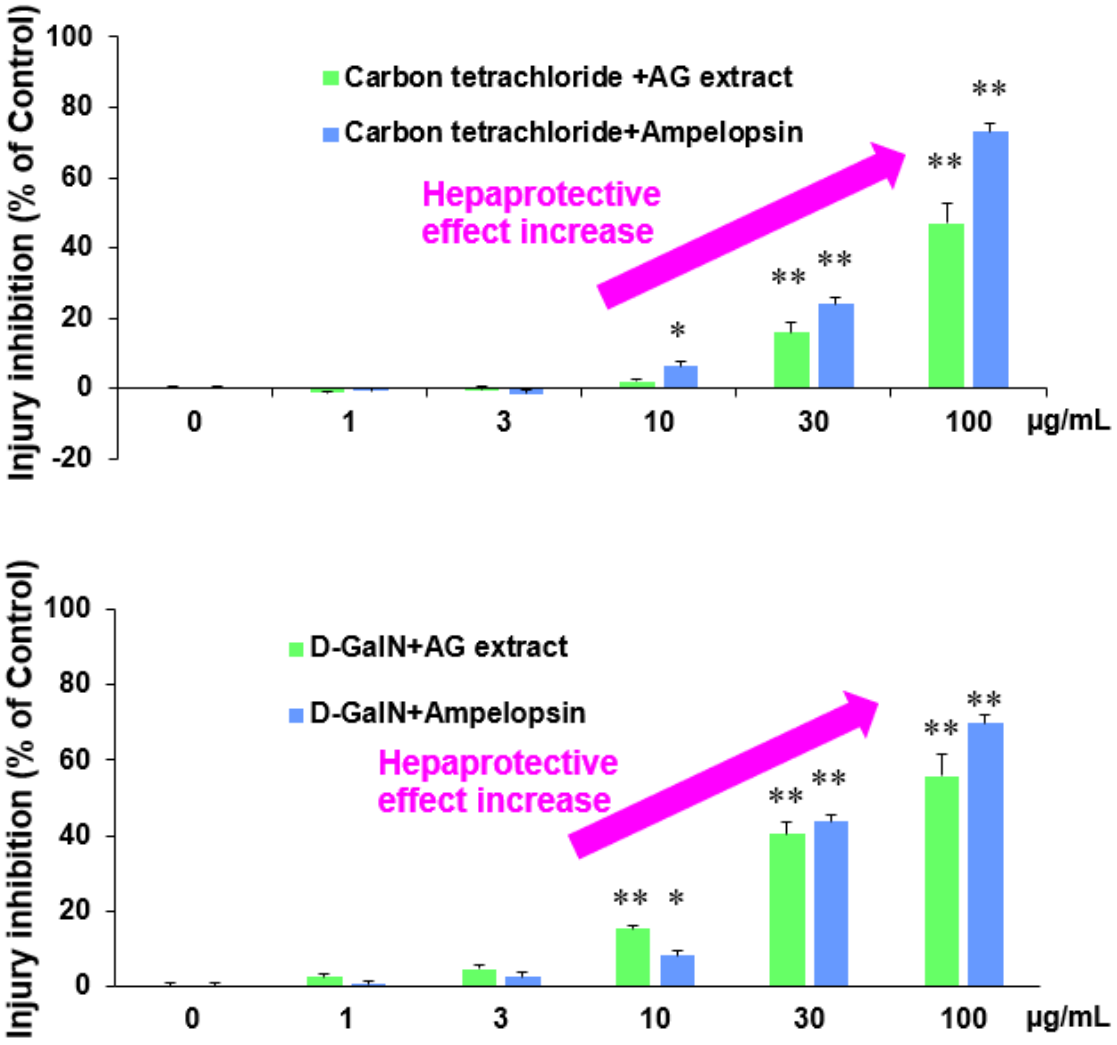



**Fig. 28** Effects of AG extract and ampelopsin on ALDH2 and CYP2E1 protein expression in the liver of alcohol diet-fed mice.
Each column represents the mean ± SE (n=3-4). Asterisks denote significant differences

from the control group at *p < 0.05.

## 4.2 *In vitro* experiments

$CCl_4$, D-GaIN and acetaminophen induced primary cultured hepatocyte injury model were also used to evaluate the effect of AG extract and ampelopsin [4, 5]. As a result, compared to the control group, AG extract and ampelopsin showed a concentration-dependent inhibitory effect on hepatocyte injury(Fig. 29).



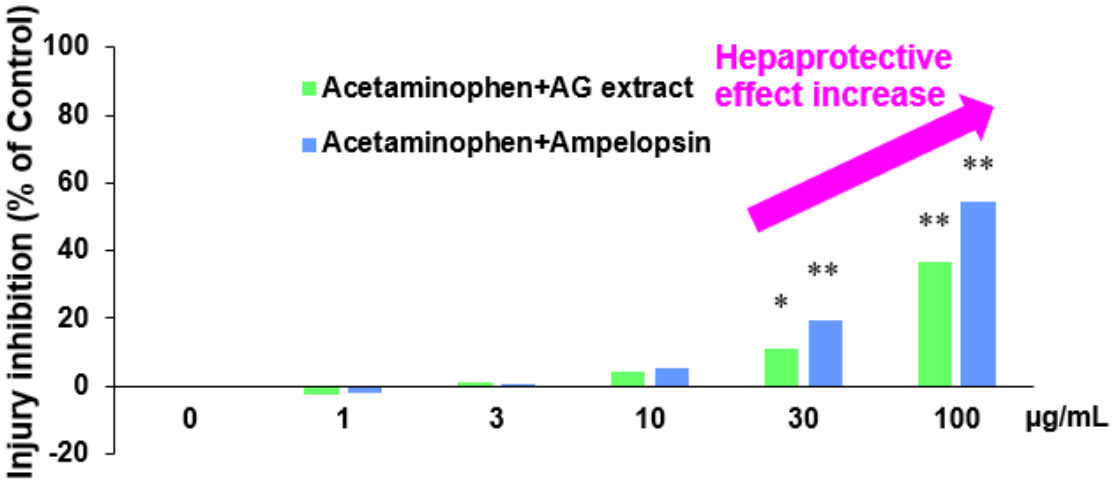



**Fig. 29** Effects of AG extract and ampelopsin on CCl4, D-GaIN and acetaminophen induced primary cultured hepatocyte injury.

Each column represents the mean with SE (n=4-6). Asterisks denote significant differences from the control group at *$p$<0.05, **p<0.01.

**References：**

1. Shimoda H, Tanaka J, Kikuchi M, Fukuda T, Ito H, Hatano T, Yoshida T. Walnut Polyphenols Prevent Liver Damage Induced by Carbon Tetrachloride and D-Galactosamine: Hepatoprotective Hydrolyzable Tannins in the Kernel Pellicles of Walnut. *J. Agric. Food Chem.* **2008**, 56: 4444-4449.
2. Bertola A, Mathews S, Ki SH, Wang H, Gao B. Mouse model of chronic and binge ethanol feeding (the NIAAA model). *Nat Protoc.* **2013**, 8: 627-637.
3. Silva J, Yu X, Moradian R, Folk C, Spatz MH, Kim P, Bhatti AA, Davies DL, Liang J. Dihydromyricetin Protects the Liver via Changes in Lipid Metabolism and Enhanced Ethanol Metabolism. *Alcohol. Clin. Exp. Res.* **2020**, 44: 1046-1060.
4. Matsuda H, Ninomiya K, Shimoda H, Yoshikawa M. Hepatoprotective principles from the flowers of Tilia argentea (linden): structure requirements of tiliroside and mechanisms of action. *Bio org Med Chem.* **2002**, 10: 707-712.
5. Moritani C, Kawakami K, Shimoda H, Hatanaka T, Suzaki E, Tsuboi S. Protective Effects of Rice Peptide Oryza Peptide-P60 against Oxidative Injury through Activation of Nrf2 Signaling Pathway In Vitro and In Vivo. *ACS Omega*. **2020**, 5: 13096-13107.

## 5. Anti-oxidant effect

Reactive oxygen species (ROS) are generated through normal metabolism. However, environmental stress such as UV exposure and oxidative stress due to modern lifestyle may increase levels of ROS. Fig. 30 showed that AG extract demonstrated strong antioxidant effect on DPPH & SOD model.

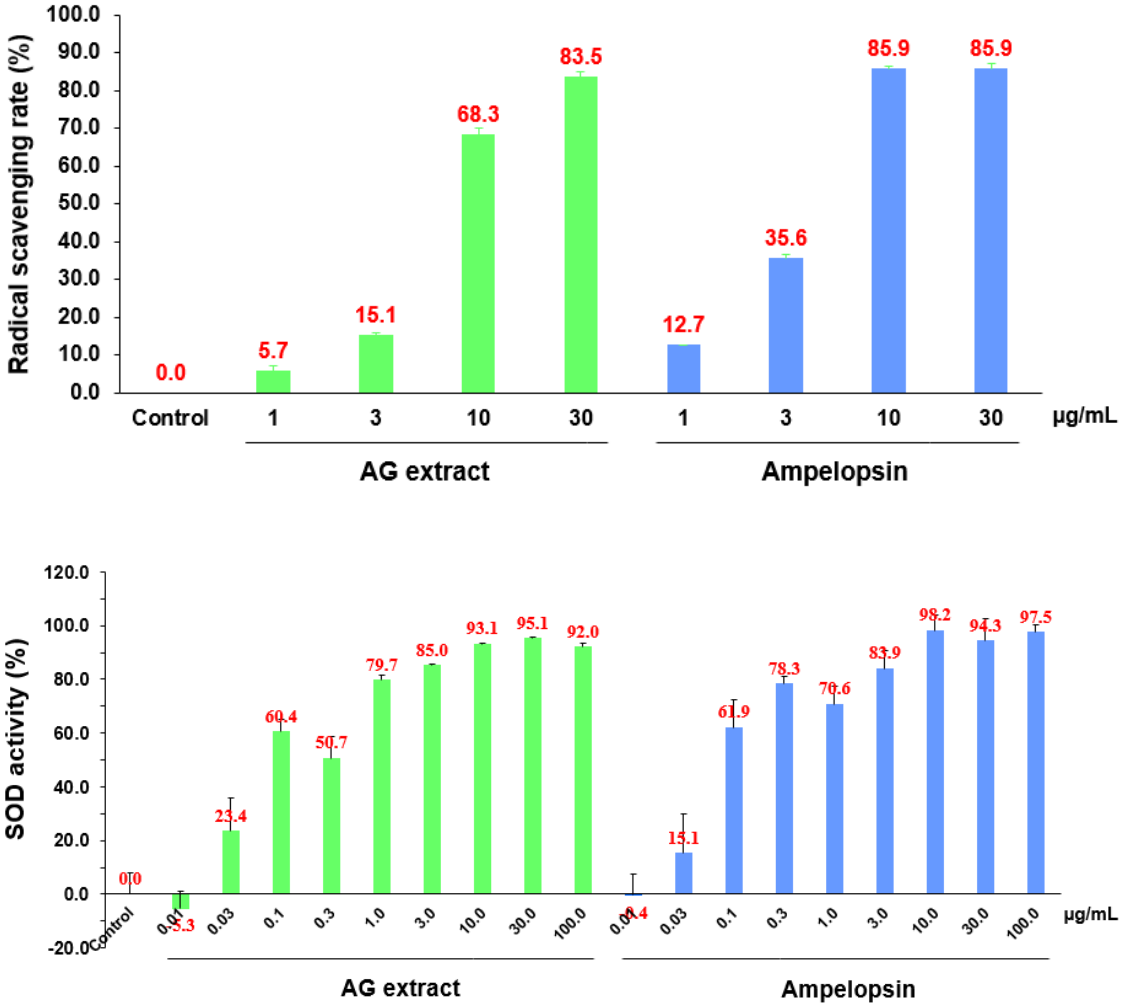



**Fig. 30** Effect of AG extract on DPPH radical scavenging model and SOD model (n=3).

# 6. Stability

## 6.1 Stability at high temperature

**Vine Tea extract-P at high temperature**

The content of Vine Tea Extract-P (containing 50% ampelopsin) was almost no decrease when heated at 100 and 120 ℃ for 2 hours.

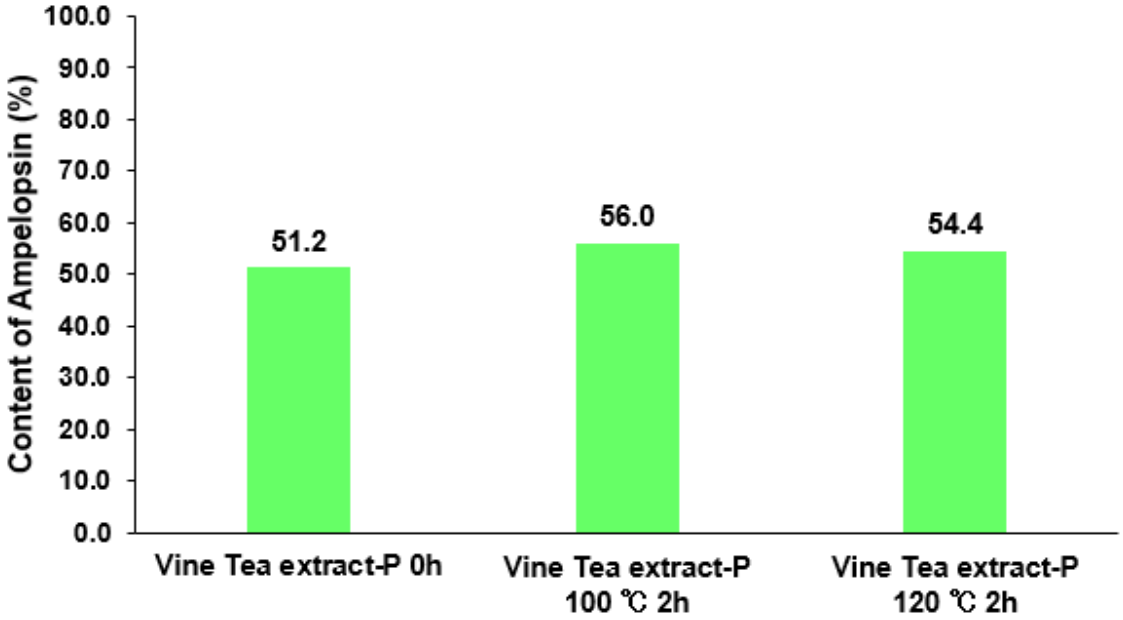



**Fig. 31** Stability of Vine Tea extract-P at high temperature

## 6.2 Acceleration test

**Long-term stability test of Vine Tea extract-P**

The content of Vine Tea Extract-P (containing 50% ampelopsin) was almost no decrease when in the acceleration condition of (40 ℃, RH 75%) for 6 months.

**Table 6**. Content of Vine Tea extract-P in the acceleration condition of (40 ℃, RH 75%)

| | **Content of Ampelopsin（%）** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 M | 2 Weeks | 1 M | 2 M | 3 M | 4 M | 5 M | 6 M |
| **Vine Tea extract-P** | **51.2** | **51.0** | **51.7** | **51.0** | **50.9** | **51.2** | **50.6** | **51.3** |

# 7. Nutritional ingredients

### Nutritional ingredients of Vine Tea extract-P

**Table 7**. Nutritional ingredients of Vine Tea extract-P (containing 50% ampelopsin)

| Items | Ingredients of 100g | Analytical method |
|---|---|---|
| 8 | **Vine Tea extract-P** | Note 1 |
| Energy | 361 kcal | Note 2 |
| Protein | 2.0 g | Kjeldahl method, nitrogen protein conversion factor: 6.25 |
| Fat | 0.4 g | Acid decomposition method |
| Carbohydrate | 89.1 g | Note 3 |
| Sugar | 85.8 g | Note 4 |
| Sodium | 45.0 mg | Atomic absorption spectrophotometory |
| Sodium chloride equivalent | 0.11 g | Sodium conversion value |
| Water | 6.7 g | Reduced pressure heating method |
| Ash | 1.8 g | Direct incineration method |
| Fiber | 3.3 g | Prosky method |

**Note 1** Analytical method:
The nutritional information of Vine Tea extract-P was analyzed according to the standard in nutrition labeling (March 30, 2015; No 139 Eishin).
**Note 2** Energy conversion factor: Protein 4, fat 9, sugar 4, dietary fiber 2
**Note 3** Calculation: 100-(water + protein + fat + ash)
**Note 4** Calculation: 100-(water + protein + fat + fiber + ash)

Test trustee : SUNATEC
Test date : April 15, 2021
Test No. : 210401563-001-01

# 8. Safety

## 8.1 Residual agricultural chemicals

Vine Tea extract-P (containing 50% ampelopsin) was screened and analyzed for residual agricultural chemicals (504 items) according to the Food Sanitation Act and Pesticides Control Act, results of the test items were lower than the allowed limits.

Test trustee : SUNATEC
Test date : April 12, 2021
Test No. : 210401563-002-01

## 8.2 Acute toxicity (LD50)

Vine Tea extract-P (containing 50% ampelopsin) was orally given to fasted ICR mice (20～25 g, 5 weeks old). After 14 days, no abnormalities and fatal event were observed at 2000 mg/kg. No abnormalities were observed under macroscopic examination upon autopsy. Thus, LD50 of Vine Tea extract-P is deduced to be > 2000 mg/kg.

## 8.3 Mutagenicity (Ames test)

Ames test was conducted to evaluate the mutagenicity of Vine Tea extract-P (containing 50% ampelopsin) using Salmonella typhimurium TA98, TA100, TA1535, TA1537 and E. coil WP2 at concentration 19.5-5,000 μg/plate. No mutagenicity was observed.

Test trustee : BoZo Research Center Inc.
Test date : June 25, 2021
Test No. : N-T7836

## 9. Recommended dosage

| Product | Effect | Dose |
|---|---|---|
| Vine Tea extract-P | Anti-obesity<br>Hepaprotective | 250 mg/day |

## 10. Application

| | Application | Effect | Dosage form |
|---|---|---|---|
| Food | Nutritional and Supplementary food | Anti-obesity<br>Hepaprotective | Hard and soft capsules, tablets etc. |

## 11. Package

| Product | Packing type | Weight |
|---|---|---|
| Vine Tea extract-P<br>(Powder for food) | Interior packing: Aluminium bag<br>Exterior packing: Cardboard box | 1 kg<br>5 kg |

## 12. Storage

Avoid high temperature and humidity, and store in a sealed state at room temperature and dark place. Please use it immediately after opening, if it needs to in opening state, be sure to dehumidify it with a hygroscopic agent.

# 13. Expression

**＜Food＞**

**Vine Tea extract-P**

Vine Tea extract, Maltodextrin

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

# PRODUCT STANDARD

PRODUCT NAME : **VINE TEA EXTRACT-P** (FOOD)

This product is a powder extracted from the leaves of vine tea (*Ampelopsis grossedentata*) with aqueous ethanol.
It guarantees minimum of 50.0 % of ampelopsin.

| | | |
|---|---|---|
| **Appearance** | Pale yellowish brown to brown powder with slightly characteristic odor. | |
| **Ampelopsin contents** | Min. 50.0 % | (HPLC) |
| **Loss on Drying** | Max. 10.0 % | (Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr) |
| **Purity Test** | | |
| **(1) Heavy Metals (as Pb)** | Max. 20 ppm | (Sodium Sulfide Colorimetric Method) |
| **(2) Arsenic (as $As_2O_3$)** | Max. 1 ppm | (Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B) |
| **Standard Plate Counts** | Max. $1\times10^3$ cfu/g | (Analysis for Hygienic Chemists) |
| **Moulds and Yeasts** | Max. $1\times10^2$ cfu/g | (Analysis for Hygienic Chemists) |
| **Coliforms** | Negative | (Analysis for Hygienic Chemists) |

**Composition**

| Ingredient | Content (Values are just a guide) |
|---|---|
| Vine tea extract | 75 % |
| Maltodextrin | 25 % |
| Total | 100 % |

| | |
|---|---|
| **Expiry date** | 3 years from date of manufacturing. |
| **Storage** | Store in a dry, ventilated location. Keep away from high temperature and sun light. |

| | |
|---|---|
| Established Date | September 9, 2021 |
| Revised Date | - |
| Specification No. | R-109WY |

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

**From product planning to OEM** - For any additional information or assistance, please contact：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ISO 22000 BUREAU VERITAS Certification

ISO 22716 BUREAU VERITAS Certification

**“The catalog was created based on academic data. For expressions of consumer products containing this product, follow the Health Promotion Law, Pharmaceutical Low, and other related laws and regulations.”**



Established Date: December 10, 2021

ORYZA OIL & FAT CHEMICAL CO., LTD.