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Full Length Research Paper

Comprehensive study of the intestinal absorption of four phenolic compounds after oral administration of *Ananas comosus* leaf extract *in vivo* and *in vitro*

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The extract of *Ananas comosus* leaf (EAL) was proved to be antihyperlipidemia and antihyperglycemia consisting of rich phenolic acids. Pharmacokinetic study showed p-coumaric acid was a rich compound detected in the mouse plasma after EAL oral administration. The aim of this study was to explore the alteration of the absorption of four principle phenolic compounds through the intestines by oral administration. Liquid chromatography-mass spectrometry (LC-MS-MS) was used to detect the phenolic compounds in EAL. The assay for the absorption of the small intestines of mice was employed *in vivo* and *in vitro*. According to our results, 1-O-p-coumaroylglycerol and 1-O-caffeoylglycerol would be converted into p-coumaric acid and caffeic acid by passing though the intestines. Caffeic acid might be transformed into a new compound with relative molecular mass of 359 in liver. The phenolic components of EAL were absorbed mainly in the form of p-coumaric acid while caffeic acid were absorbed through the intestines. p-Coumaric acid and caffeic acid are considered the active components of EAL in the body.

Key words: *Ananas comosus*, phenolic acid, absorption, mouse, metabolism.

INTRODUCTION

Ananas comosus (Linn.) Merr or pineapple, which originated from Brazil, is one of the most popular tropical and subtropical fruits in the world. The current major pineapple-producing countries include Thailand, Philippines, Indonesia, Vietnam, Brazil, South Africa, United States, and southern China. Aside from being a delicious food, pineapple has been used as a folk medicine for digestion and diarrhea (Borrelli et al., 2011). By recent study, the extract of *A. comosus* leaves (EAL) have been detected with richness of polyphenols, which are known as antimicrobial agents (Ma et al., 2007; Gazzani et al., 2011). Our previous study showed that EAL has comprehensive effects on diabetic- dyslipidemic rats (Xie et al., 2005). It was shown that EAL has significant anti-diabetic, anti- dyslipidemic, and anti-oxidative effects as well as insulin sensitivity improving in animal models (Xie et al., 2006, 2007). However, studies on the metabolism of polyphenols in EAL by oral administration are few.

Polyphenols, a large group of natural antioxidants, are generally involved in defense against ultraviolet radiation or aggression by pathogens. Epidemiological studies and associated meta-analyses strongly suggested that long term consumption of plants rich in polyphenols offer protection against hypertensive (Rodrigo et al., 2012), oxidants (Attia et al., 2013), dermatophytes (Tao et al.,

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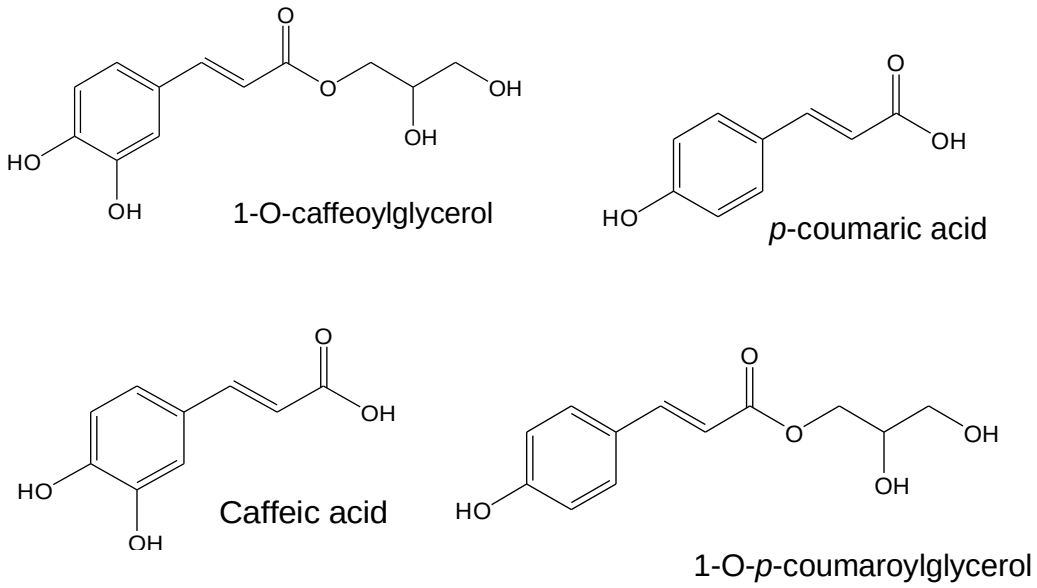

Figure 1. Chemical structure of four compounds.

2013), anxiolytic activity (Malik et al., 2013), development of lung cancer, cardiovascular disease, diabetes and neurodegenerative diseases (Pandey et al., 2009). Here, we present the absorption and biological effects of phenolic compounds after oral administration of EAL in the context of relevance to hypolipidemic and hypoglycemic.

We previously reported that EAL is rich in phenolic acids, including *p*-coumaric acid, caffeic acid, 1-O-*p*-coumaroylglycerol, and 1-O- caffeoylglycerol (Figure 1) (Ma et al., 2007; Wang et al., 2006). The most abundant and stable component of EAL is *p*-coumaric acid, which is the main form of phenolic component presented and excreted *in vivo*. p-Coumaric acid has been reported to inhibit the growth of Staphylococcus aureus, Shigella, and Escherichia coli (Pereira et al., 2007). Animal experiments have demonstrated that the lipid-lowering effect of *p*-coumaric acid manifests by protecting low-density lipoprotein cholesterol from oxidation (Morais et al., 2009). Therefore, it is used as a bioactive marker for quality control of EAL (Zang et al., 2000). The kinetic behavior of p-coumaric acid in mouse plasma has been detected after oral administration of EAL. *p*-Coumaric acid was found with quick absorption (T_{max} 0.062 h) and short half-life time ($t_{1/2}$ 0.023 h) (Meng et al., 2006). Four major ingredients were able to be detected in the plasma, bile and urine (data are not shown). However, the ratio of the ingredients was altered in these samples, p-coumaric acid became more and the other three ingredients became less. It was mentioned that there stayed a metabolism during the ingredients of EAL absorbed just through the intestines. Therefore, the absorption of the four ingredients of EAL after oral administration needs to be studied. In this study, HPLC and LC-MS-MS were used to detect and identify the phenolic compounds of EAL. The absorption by pass through the intestines *in vivo* and *in vitro* was used as model. The microsome incubation of live and small intestines was used to confirm the metabolites and bio-transformation of the four compounds.

MATERIALS AND METHODS

Chemicals and reagents

p-Coumaric acid (trans-*p*-hydroxycinnamic acid) standard (98% purity) was purchased from Sigma (U.S.). 1-O-*p*-Coumaroylglycerol, caffeic acid, and 1-O-caffeoylglycerol standards were provided by Dr. Wei Wang. EAL with batch number 110801 was produced in our laboratory, containing 0.14% of 1-O-*p*-coumaroylglycerol, 1.31% of caffeic acid, 0.19% of 1-O-caffeoylglycerol and 1.98% *p*-coumaric acid by high performance liquid chromatography (HPLC) determination. Methanol and acetonitrile (HPLC grade) were purchased from Xinhua Special Reagent Factory, Tianjin, China.

Animals

Male ICR mice weighing 23-25 g and male Wistar rats weighing 230-250g were purchased from Vital River Laboratories (Beijing, China). The animals were raised in an environment with 60 ± 5% humidity at 25°C under a 12 h dark/light cycle. The animals were fasted overnight before the experiment. All the animals and the experimental procedures were approved by the Animal Welfare and Ethics Committee of Tsinghua University and the Institutional Animal Care and Use Committee of Tsinghua University.

Chromatographic system and conditions

Phenolic acids were detected using HPLC for quantity and LC-MS-MS for indentify of four compounds in EAL. The HPLC system were employed consisting of two 515 pumps, a 2487 UV–vis detector, a 40-µl-injection loop, and an Empower 2 workstation (Waters, U.S.)

for data collection. Sample analysis was carried out by applying a 10 µl volume to an ODS-3 C18 reversed-phase column (5 mm, 150 × 4.6 mm) and detected at 310 nm (1-O-p- coumaroylglycerol and p-coumaric acid) and 324 nm (1-O-caffeoylglycerol and caffeic acid). The mobile phase [0.1% phosphate acid, pH:3 water–acetonitrile (88:12, v/v) filtered through a 0.45 µm millipore filter and degassed prior to use] was utilized at a flow rate of 1 mL/min with a constant temperature (25°C).

A liquid chromatography/ linear ion trap mass spectrometry (LC-MS) system (Agilent 1200/6340). The LC equipment (Agilent 1200) comprised of MS pump (Agilent 6340), an autosampler with a 10 µL loop. This was interfaced with an Ion trap mass spectrometer fitted with an ESI source and operating in zoom scan mode for the accurate determination of parent ion m/z, MS2 mode to abtain fragment ion m/z. MS operating conditions (negative ion) had been optimized with a nebulizer pressure of 15.0 psi, a capillary voltage of 3.5 kv, a dry gas flow rate of 8 L/min, and a dry temperature 350°C. Sample analysis was conducted by using a C18 reverse-phage column (0.5 µm; 4.6 × 150 mm, Rainbow, China) and detected at 310 nm. The mobile phase was water-acetonitrile (containing 0.1% formic acid, pH 3.0; 86:14, v/v) filtered through a 0.45 µm filter membrane used at a flow rate of 0.2 mL/min and room temperature (25°C).

Experiment procedures

Kinetic behavior and blood sample preparation

After overnight fast, the mice were orally administered with EAL aqueous solution at single dose of 300 mg/kg. At the time points (15, 30, 60, 120 and 240 min after introgastric administration), blood samples were collected and transferred to a heparinized Eppendorf tube and centrifuged at 4500 rpm for 10 min. Plasma (400 µL) was taken and mixed with 4 mL methanol (1:10, v/v). This mixture was oscillated for 30 s and centrifuged at 12000 rpm at 4°C for 10 min. The supernatant was collected and dried at room temperature. Meanwhile, the small intestines were taken and washed with 5 mL methanol. The eluate ion was transferred in a vial and dried at room temperature. The residue of blood samples was dissolved in 100 µL methanol, whereas that of the small intestine solution was dissolved in 500 µL methanol. 10 µL of each sample was used for HPLC analysis.

Absorption through the intestines *in vivo* and *in vitro*

In vivo

Male Wistar rats were anesthesiaed by injection of urethane (1 g/kg, i.p.) and kept in a plat with supine position. The small intestines were got out in a normal saline incubation (37°C). After the intestines were merged into the incubation 10 min, 2 ml normal saline sample was taken as negative control. Then, EAL was given by oral administration. 2 h later, 2 ml normal saline sample was taken for the compounds determination. The samples were all stored at -80°C until to detect.

In vitro

After anesthesiaed by injection of urethane, the intestines of rats were scarded as small intestines and large intestines (colon). The small intestines were further separated as three parts, duodenum, jejunum and ileum. All parts for the experiment were fitted in 2 cm long. All parts of the intestines were turn down from inner to outer. The bag was taken by ingaturing the both ends of the intestines. After that, the bags from the intestines were taken into the incubation liquid containing EAL at 37°C for 30 min. The bags were taken out and washed in normal saline three times. The absorption liquid was got from the bag and stored at -80°C until to detect. Normal saline was used as a negative control.

Conversion experiment *in vitro*

The mice were killed by cervical dislocation, and the liver and small intestines were taken out for the microsome solution containing metabolite enzymes (Ren et al., 2009). The homogenates were centrifuged at 15 000 × g for 10 min at 4°C. 7 ml of supernatants was collected and 1.25 ml of 52 mM $CaCl_2$ was added. Tubes were gently shaken for 5 s and allowed to stand in an ice bath for 15 min. The solution was then centrifuged at 25 000 × g for 15 min. Pellets were finally resuspended in 0.3 ml of 0.1 M potassium phosphate buffer containing 20% glycerol. The microsome solution was aliquoted and stored at -80ºC until use.

The quantity of protein in microsome solution was acquired by total protein (TP) assay kit (Zhongsheng Biotechnology Company, Beijing, China) and the concentration of protein was set at 7.5 mg/ml. From the solutions of both liver and small intestine, 100 µL samples were taken, and EAL (10 µg) was added in. After the mixture was incubated at 37°C for 30 min, 1 mL cold methanol was added to stop the reaction. The sample handling for this experiment was the same as with the plasma sample.

Caffeic acid was added to 100 µL microsome incubations of liver and small intestine. The mixture was incubated at 37°C for 30 min, and 1 mL cold methanol was added to stop the reaction. Then, the sample handling was the same as with the plasma samples.

Data analysis

All data are expressed as mean ± S.D. Data were statistically analyzed by using one-way analysis of variance (ANOVA) with F value determination. Values obtained at $P<0.05$ were considered statistically significant. All statistics were carried out by using the software for Office2007 (Microsoft, U.S.). The content of four compounds was determined by their standard curve and the equations. Ratio of each compound among these compounds was conducted by the equation, [=content of each compound / (sum of these four compounds) × 100%].

RESULTS

HPLC and LC/MS/MS analysis

By HPLC, four compounds of EAL, 1-O-caffeoylglycerol, caffeic acid, 1-O-*p*- coumaroylglycerol and *p*-coumaric acid, were shown in accordance with the standard compounds (Figure 1 and 2). Their retention times were 4.651 min (1-O- caffeoylglycerol), 5.276 min (caffeic acid), 8.227 min (1-O-*p*-coumaroylglycerol) and 9.335 min (*p*-coumaric acid). There were no interfarance in the detecting time. All the compounds were identified by LC/MS/MS (Figure 3). The standard curves of four compounds were conducted and the equations for quantity were as follows: 1-O-caffeoylglycerol: y (ng)= 20000x +11512 (r^2=0.9976); Caffeic acid: y (ng)= 3322.19x +1614.9 (r^2=0.9949); 1-O-*p*-coumaroylglycerol: y (ng)= 2781x+ 4366.4 (r^2=0.9979) and *p*-coumaric acid: y (ng)=5586x+14290 (r^2=0.9998).

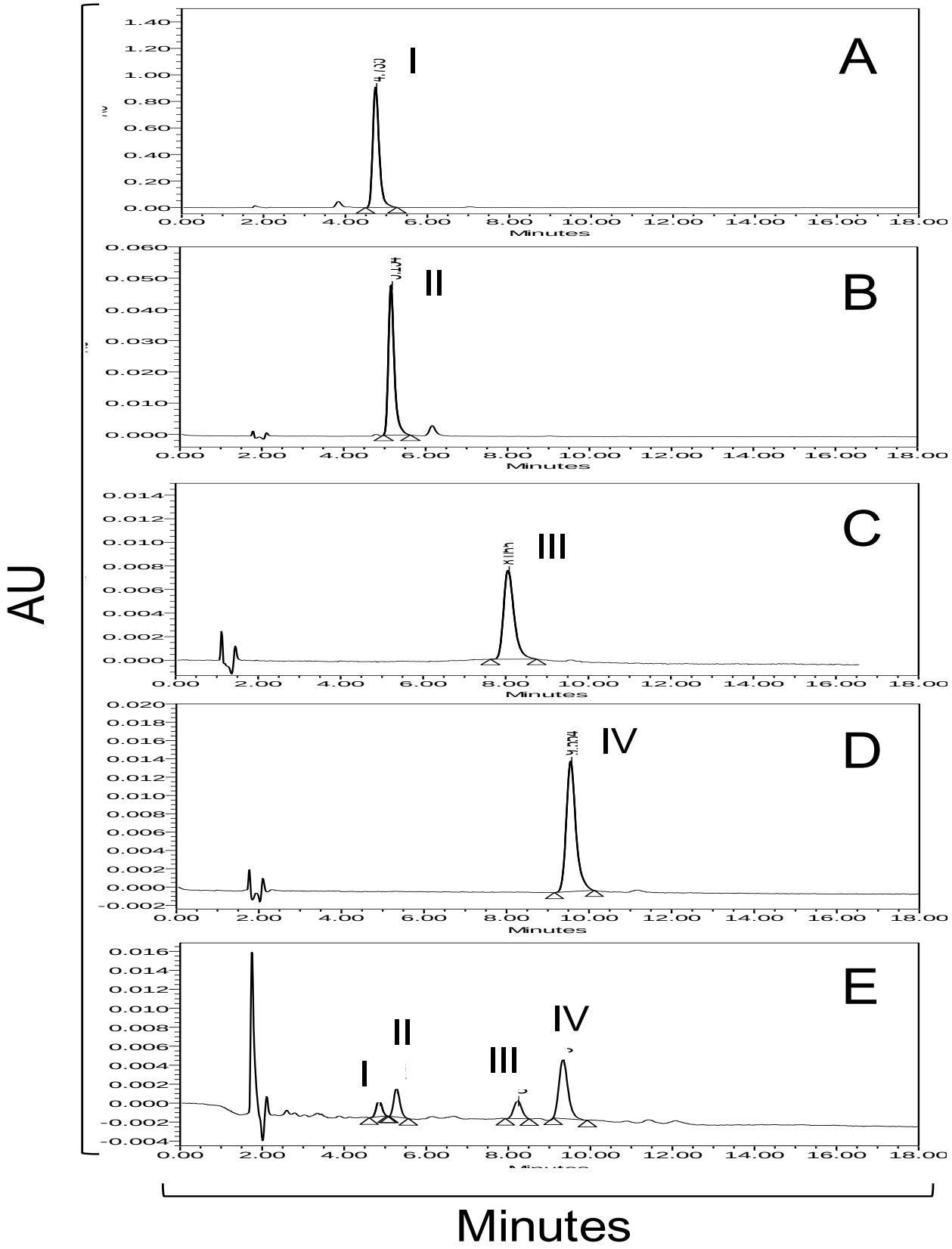

Figure 2. Typical chromatograms for the conversion of extract of *A. comosus* leaf (EAL) in samples. Chromatograms of (A) 1-O-caffeoylglycerol (I), (B) caffeic acid (II), (C) 1-O-*p*- coumaroylglycerol (III), (D) *p*-coumaric acid (IV) and (E) four compounds of EAL.

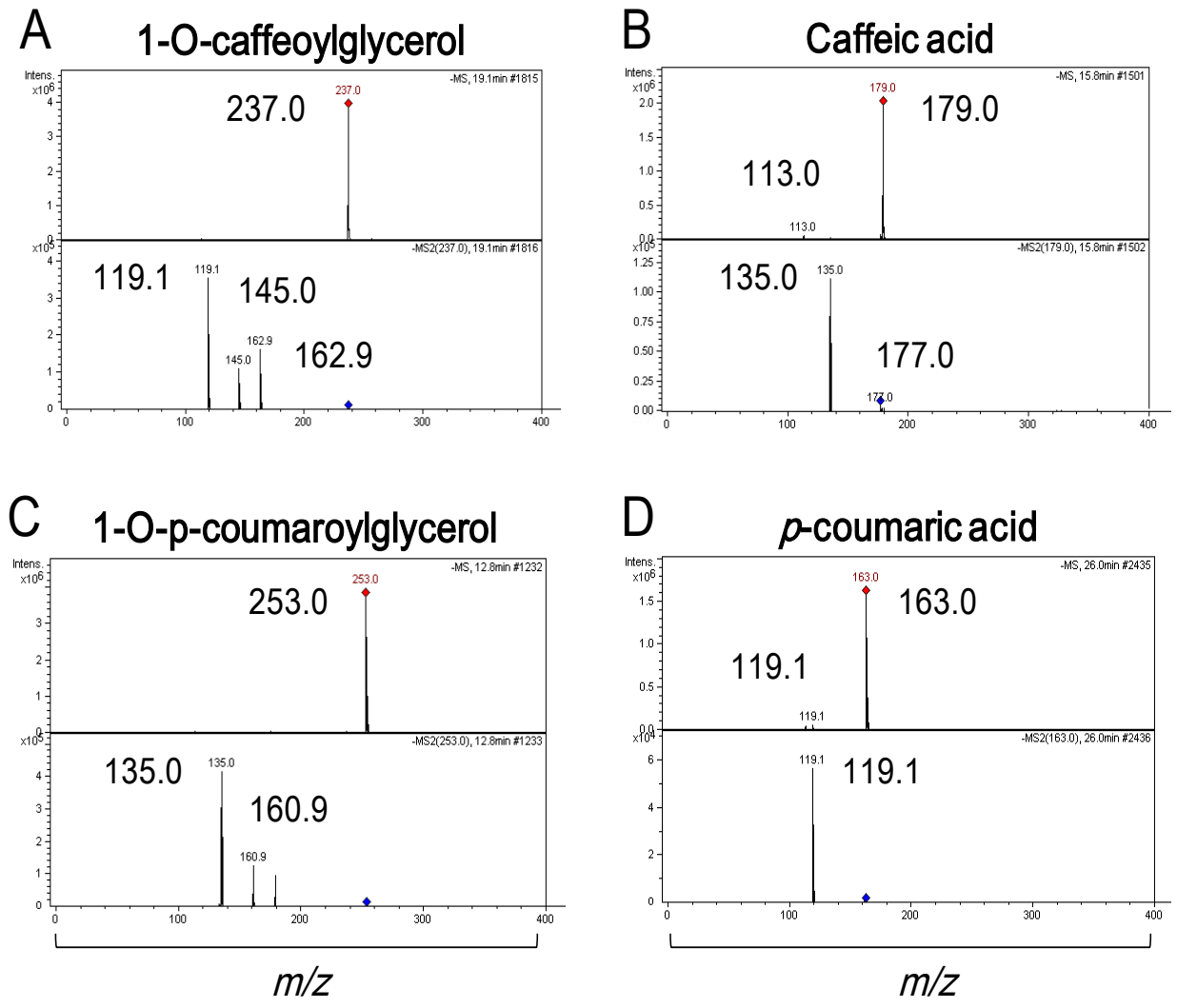

Figure 3. Mass chromatography of the four compounds. A: 1-O-caffeoylglycerol; B: caffeic acid; C: 1-O-*p*-coumaroylglycerol; D: *p*-coumaric acid.

Kinetic behavior and the compounds of EAL in mice plasma

After 15 min oral administration of EAL, *p*-coumaric acid was detected with higher peak concentration. Thereafter, it came down gradually near to the bottom at 240 min (Figure 4A). The concentration of four compounds of EAL in plasma at the time of 15 and 30 min was detected. The ratio of *p*-coumaric acid to the total compounds was highest, implying *p*-coumaric acid was maintained in major (Figure 4B).

Intestinal absorption of the compounds of EAL *in vivo* and *in vitro*

The absorption of four compounds in EAL was not adequate in rat small intestines. Generally, 1-O-caffeoy and 1-O-*p*-comar were found to be less than caffeic acid and p-coumaric acid. The content of *p*-coumaric acid was found to increase highly than that of three compounds (Figure 5A). The ratio of p-coumaric acid in the total content of all the four compounds became larger up to 89.36%. The ratio of caffeic acid was found to decrease to 9.61% compared to that of 35.37% of EAL. The ratio of 1-O-*p*-comar was 1% down from 4.89% of EAL. The ratio of 1-O-caffeoy was 0.03% down from 3.81 of EAL. Therefore, it is clear that the good absorption of p-coumaric acid was confirmed by small intestines (Figure 5B).

By the assay of different part of small and large intestines, the four compounds was found to be able to absorbed effectively through the duodenum and jejunum. The available absorption of ileum was weaker than duodenum and jejunum. Colon was poor for the absorption of four compounds (Figure 6). In duodenum,

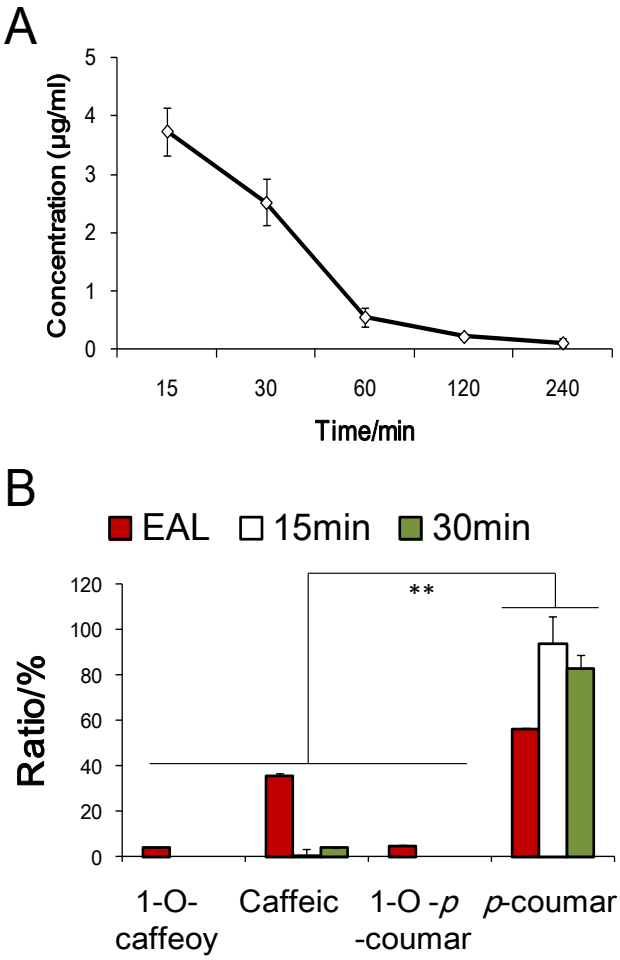

Figure 4. Kinetic concentration of *p*-coumaric acid (A) and the ratio of four compounds (B) in blood after oral administration of extract of *A. comosus* leaf (EAL) in mice. 1-O- caffeoy :1-O-caffeoylglycerol; Caffeic: caffeic acid; 1-O -*p* –coumar: 1-O -*p* –coumaroylglycerol; *p*-Coumar: *p*-Coumaric acid. The ratio of *p*-coumaric acid either in 15 min blood sample and or in 30 min blood sample is higher than that of other three compounds (F_{15min}, 3,8 =431.7, P<0.001; F_{30min}, 3,8 =80.2, P<0.001). Each value represents the mean±S.D. from three independent mice (n=3). ** *P*<0.01.

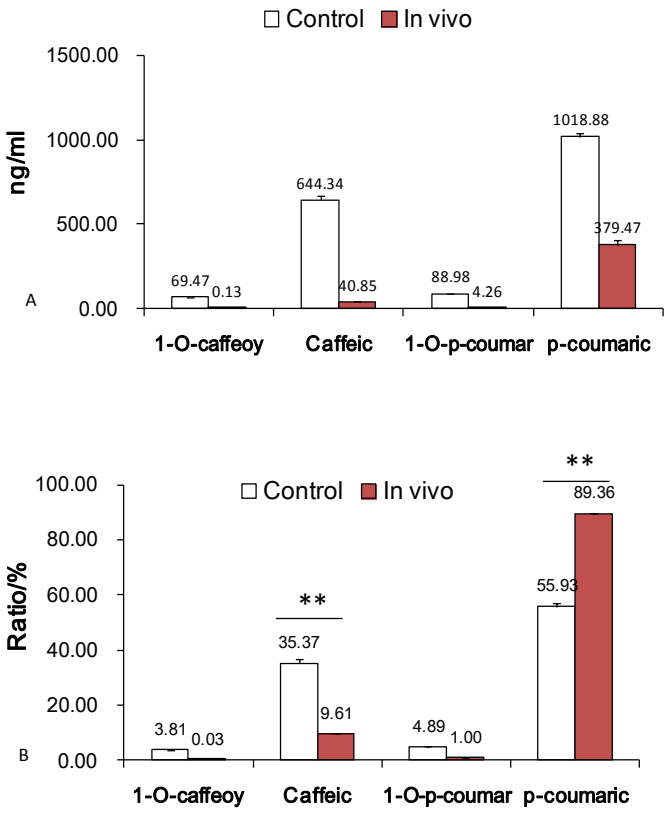

Figure 5. The absorption of the four compounds through the small intestines after extract of *A. comosus* leaf (EAL) oral administration in rats. F (3,8)=463.84, *P*<0.001. Each value represents the mean ± S.D. from three independent samples (n=3). ** *P*<0.01 *v.s.* the control groups.

the absorbed ability of *p*-coumaric acid was stronger than in the other two parts of small intestines, in which its ratio nearly equated to that of EAL (Figure 7A). The ratios of caffeic acid to the total compounds in jejunum and ileum were higher than that of EAL (Figure 7B and C). The ratio of both 1-O-caffeoylglycerol and 1-O -*p* –coumaroylglycerol in the parts of small intestines were similar to that of contral (EAL). In colon, the ratio of 1-O -*p* –coumaroylglycerol in the total compouns was decreased relatively to that of EAL (Figure 7D).

Conversion of the compounds of EAL

During the study, some of the phenolic acids could not be detected in small intestine solution and plasma. We hypothesized that other processes remained during the absorption. We therefore conducted a conversion experiment of EAL *in vitro*. After the microsome incubation, the contents of four compounds were altered. 1-O-caffeoylglycerol and 1-O-*p*- coumaroylglycerol decreased in both liver and small intestines compared to the control of EAL (Figure 8A and C). Caffeic acid in liver incubation decreased compared to that of control samples. There was no difference of caffeic aicd in the small intestines and coutrol groups (Figure 8B). The content of *p*-coumaric acid in both liver and small intestines incubations were higher than that of control groups (Figure 8D).

Getting the four ingredients together as total content, the ratio of each compound to the total content was calculated. Figure 9 shows the ratios of four compounds of EAL, microsome in liver and small intestines. Two analogues of 1-O-caffeoylglycerol and 1-O-*p*-coumaroylglycerol showed little ratio to total content compared to EAL. Speculating, caffeic acid in liver incubation was little ratio to total content distinctly implying there must be a motabolism of caffeic acid in

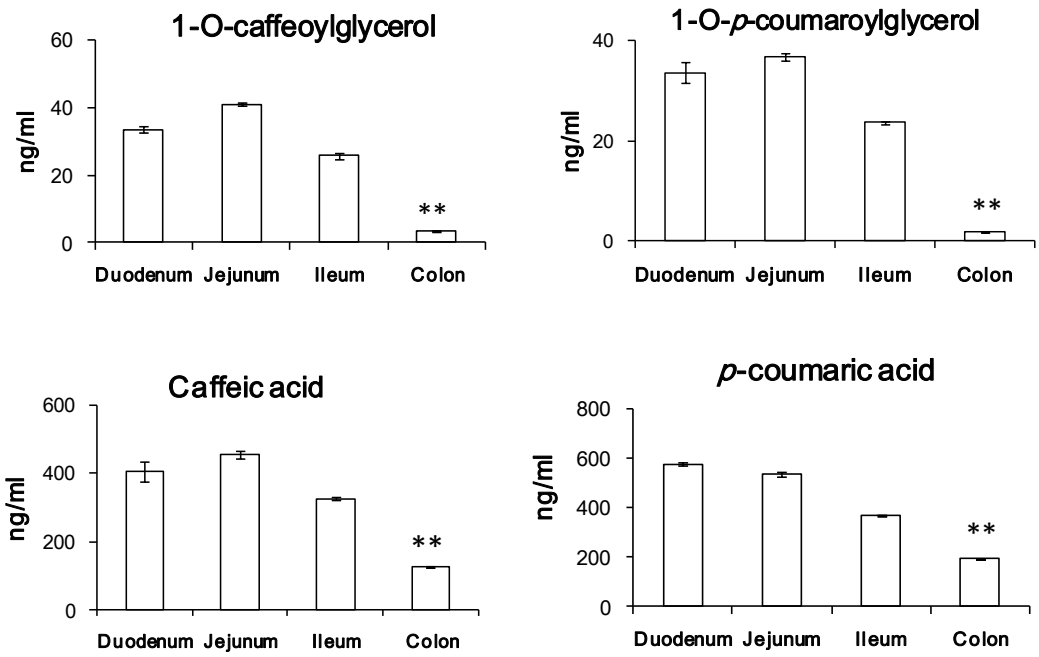

Figure 6. The absorption of the four compounds of extract of *A. comosus* leaf (EAL) through the different sizes of rat intestines. In small intestines, three parts were separated as: Duodenum, jejunum and ileum. Colon means large intestine. 1-O-caffeoylglycerol: $F(3,8)=1573.68$, $P<0.001$. Caffeic acid: $F(3,8)=219.83$, $P<0.001$. 1-O -*p* -coumaroylglycerol: $F(3,8)=12477.3$, $P<0.001$. *p*-Coumaric acid: $F(3,8)= 2926.23$, $P<0.001$. Each value represents the mean ± S.D. from three independent samples (n=3). ** $P<0.01$ v.s. the rest three parts of the small intestines.

Liver Figure 10. The ratio of *p*-coumaric acid in liver and small intestines was increased compared to EAL, suggesting there must be a biotransformation in small intestines while the ingredients were absorbed through the intestines, specially 1-O-*p*-coumaroylglycerol (Figure 9).

LC-MS-MS results supported our hypothesis. After incubation with the microsome incubation of both liver and small intestines, 1-O-*p*- coumaroylglycerol and 1-O-caffeoylglycerol were found to convert into *p*-coumaric acid and caffeic acid, respectively. It is easy to distinguish 1-O-*p*-coumaroylglycerol and 1-O-caffeoylglycerol by its MS base peak at m/z~237 and 253, supported by strong MS2 ions at m/z~119.1, 162.9 and 135, 160.9, respectively. The ion at m/z~163 is characteristic for *p*-coumaric acid, supported by strong MS2 ions at m/z~119.1. The ion at m/z~163 is characteristic for caffeic acid, supported by strong MS2 ions at m/z~135 (Fig.10). All these indicated that 1-O-*p*-coumaroylglycerol and 1-O-caffeoylglycerol were metabolised to *p*-coumaric acid and caffeic acid, respectively.

Metabolism of caffeic acid

By adding caffeic acid standard with the microsome incubation of liver and small intestine, the content of caffeic acid attenuated after the microsome incubation of liver comparing to normal saline incubation. There was no difference of caffeic acid between normal saline and small intestine incubation (Figure 11A).

Quasi-molecular ion [M−H]− of caffeic acid standard at retention time 15.8 min was 179 with product ion 135. One metabolite at the retention time of 14.4 min was found with Quasi-molecular ion [M-H]- 359. The MS2 of the metabolite included 135 and 179, indicating it was from caffeic acid. The product ions of it included 135 [M−H–Glucuronide–CO2]−, 179 [M−H– Glucuronide]−, prototype of CA (Figure 11B). All that suggested caffeic acid was glucuronized conjugate in liver microsome (Zhang et al., 2008).

This result suggested that caffeic acid might be transformed into a new compound with the effect of the microsome incubation of liver. The metabolism of caffeic acid occurred in the microsome incubation of liver but not small intestine, this might due to the different metabolic enzymes present in the liver and small intestine. All the transformation of the ingredients after oral administration of EAL is displayed in Figure 12.

DISCUSSION

In this work, we proved that phenolic acids in the extract of *A. comosus* leaf (EAL) can be absorbed in intestines

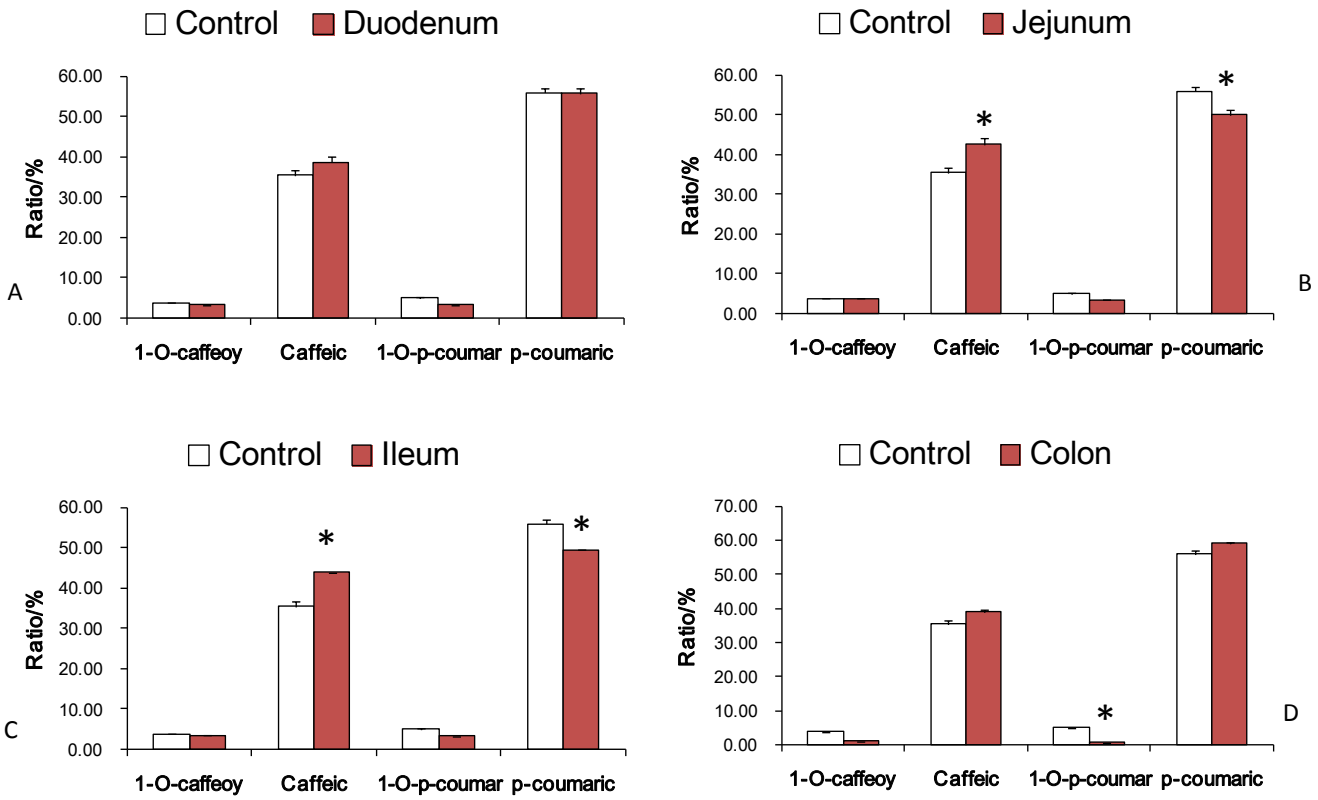

Figure 7. Ratio to the compounds through the different sizes of the rat intestines *in vitro*. 1-O-caffeoy, 1-O-caffeoylglycerol; Caffeic, caffeic acid; 1-O -*p* –coumar, 1-O -*p* –coumaroylglycerol; *p*-Coumaric, *p*-coumaric acid. Each value represents the mean ± S.D. from three independent samples (n=3). * *P*<0.05 *v.s.* the control groups.

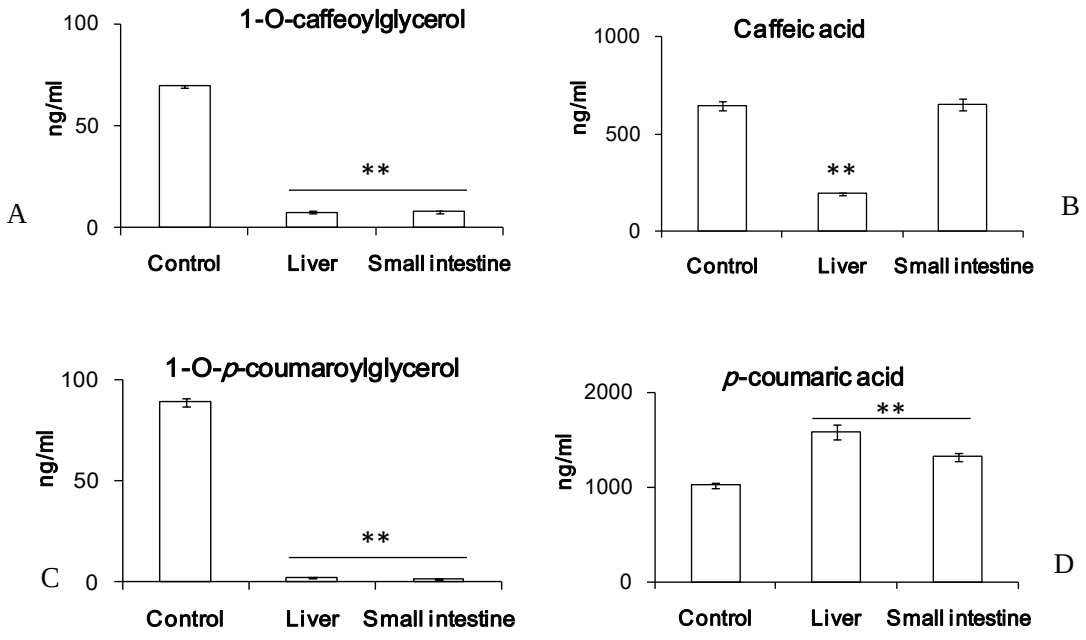

Figure 8. Concentration of four compounds in extract of *A. comosus* leaf (EAL) (1 mg/ml) in the incubation of normal saline and the microsomes of both liver and small intestines. 1-O-caffeoylglycerol: F(2,6)=5836.48, *P*<0.001. Caffeic acid: F(2,6)=439.53, *P*<0.001. 1-O-*p*-coumaroylglycerol: F(2,6)=5673.44, *P*<0.001. *p*-Coumaric acid: F(2,6)=81.72, *P*<0.001. Each value represents the mean ± S.D. from three independent samples (n=3). ** *p*<0.01 *v.s.* the control groups.

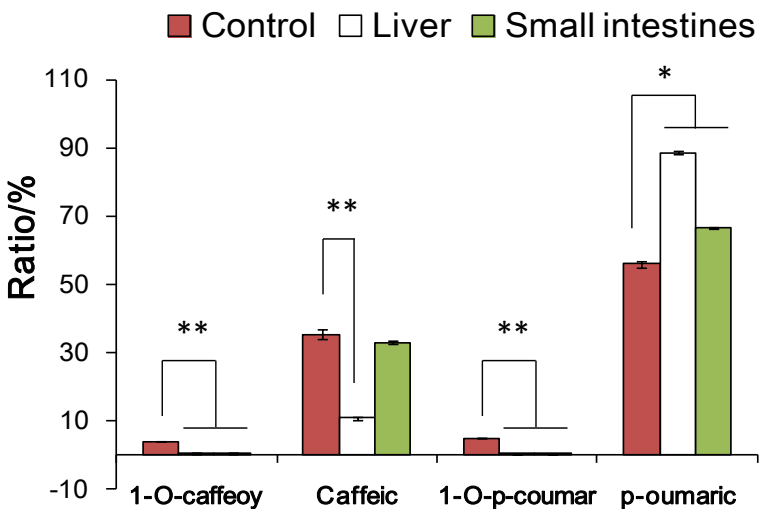

Figure 9. Ratio of each compound among these four compounds after incubation *in vitro*. Each value represents the mean ± S.D. from three independent samples (n=3). * $P<0.05$, ** $P<0.01$, *v.s.* the control groups.

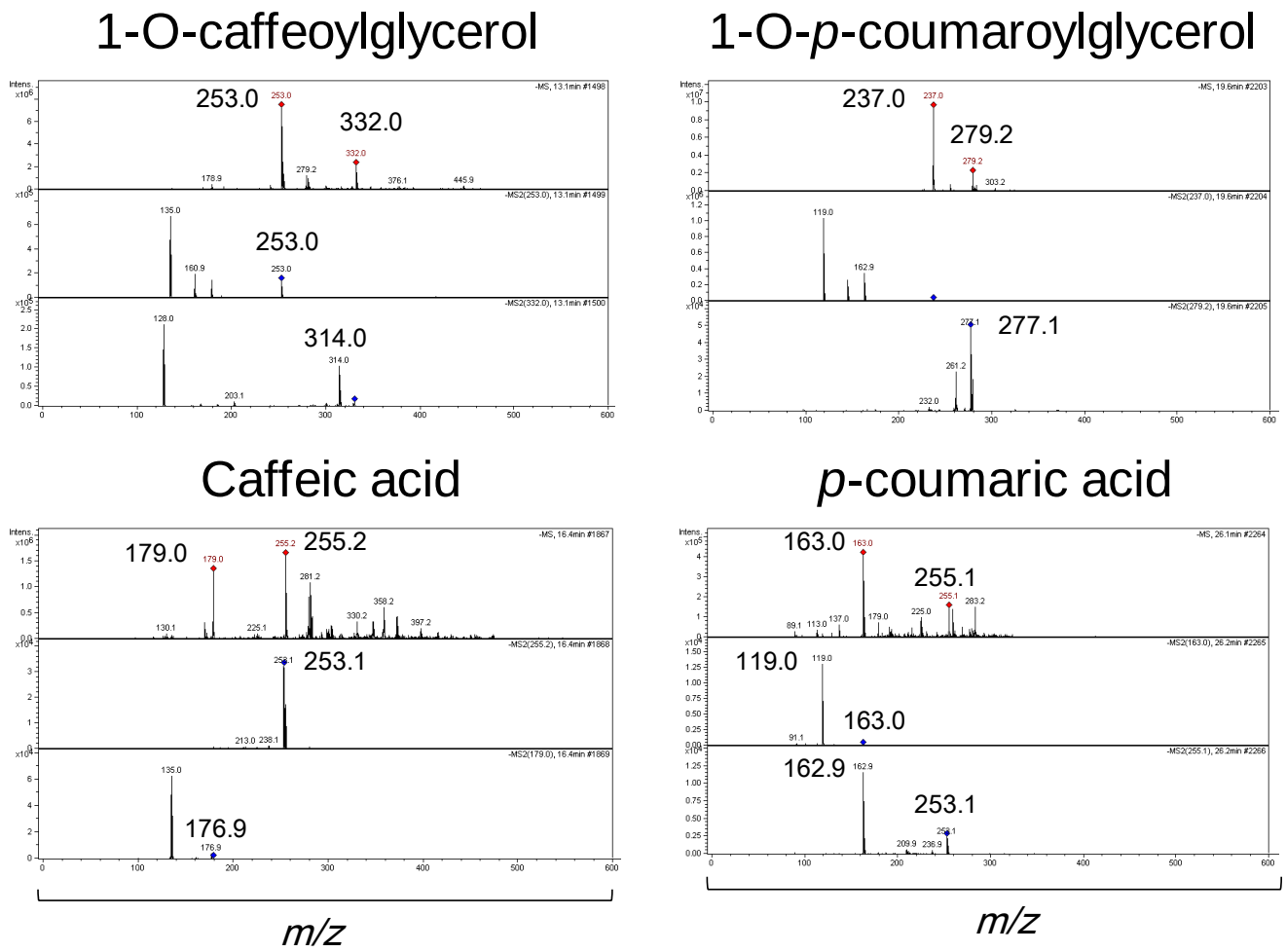

Figure 10. Liquid chromatography–tandem mass spectrometry (LC-MS) of four compounds of EAL after incubation in the liver microsome solution.

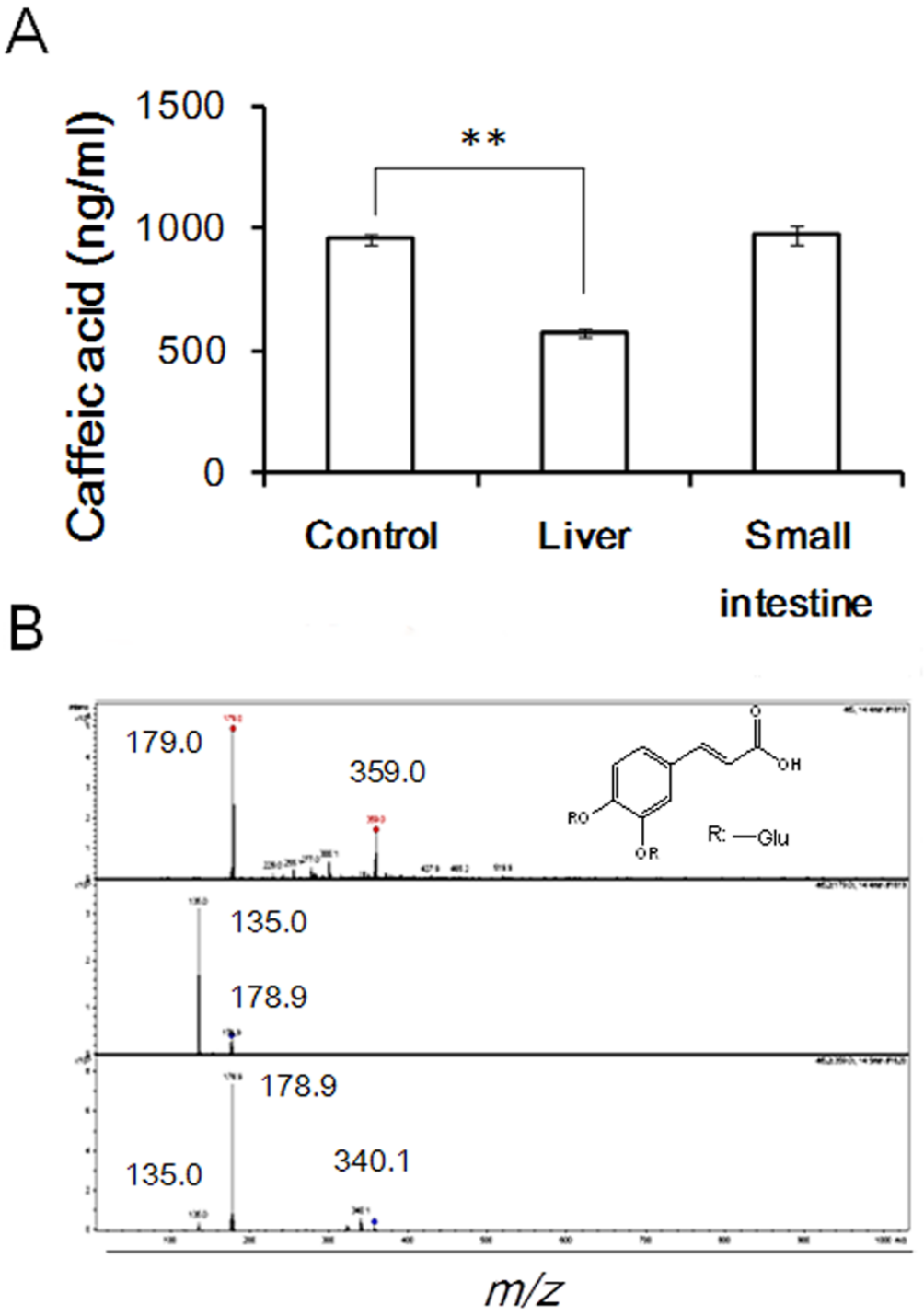

Figure 11. Contents of caffeic acid with normal saline (control) and microsome incubations of liver and small intestine (A) and liquid chromatography–tandem mass spectrometry (LC-MS) of the metabolite of caffeic acid in liver microsome (B). (A): F (2, 6) = 243.78, *P*<0.001. Each value represents the mean ± S.D. from three independent samples (n=3). ** *P*<0.01, *v.s*. the control groups.

after oral administration. There existed biological transformation when the phenolic acids were absorbed into the bloodstream by passing through the intestines. 1-O-*p*-coumaroylglycerol and 1-O-caffeoylglycerol could be converted into *p*-coumaric acid and caffeic acid. *p*-Coumaric acid and caffeic acid were the master and active components in blood after oral administration of EAL. Besides, caffeic acid could be converted into a

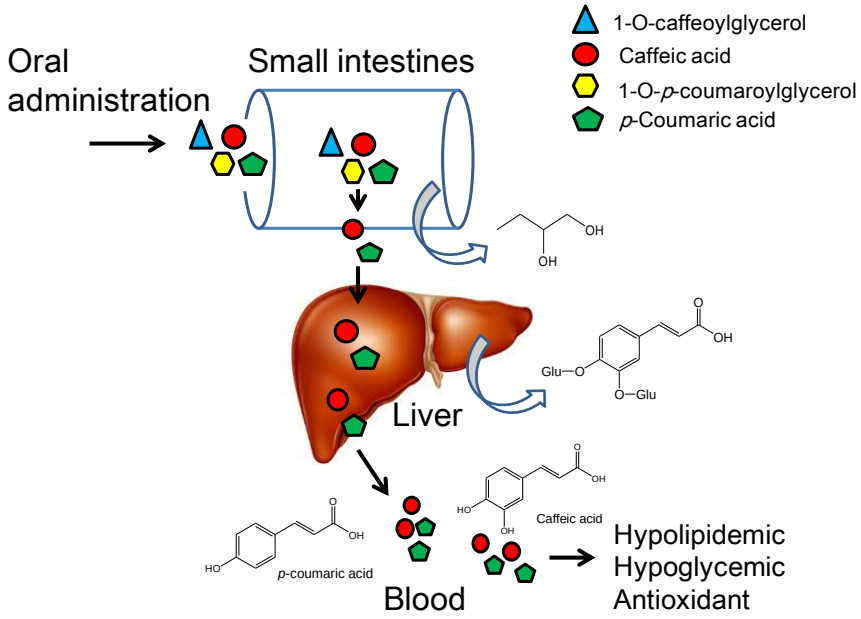

Figure 12. The schematic explanation of four ingredients after oral administration of the extract of *A. comosus* leaf. During passing through small intestines, 1-O-caffeoylglycerol converted to caffeic acid and 1-O-*p*-coumaroylglycerol converted to *p*-coumaric acid. When passing through liver, caffeic acid further transformed into a new metabolite, 3,4-O- caffeoylglucuronide.

metabolite in liver during this process.

The phenolic acids in *A. comosus* leaves are mainly *p*-coumaric acid, caffeic acid, 1-O-*p*-coumaroylglycerol, and 1-O-caffeoylglycerol. Throughout our experiments, we found that there were not other components in the plasma besides *p*-coumaric acid and caffeic acid after oral administration of EAL. We proved that there existed conversion of *p*-coumaric acid and caffeic during the absorption after oral administration of EAL. 1-O-*p*-coumaroylglycerol and 1-O-caffeoylglycerol could convert into *p*-coumaric acid and caffeic acid, respectively. Caffeic acid might be transformed into a new compound which was in agreement with previous report (Martignoni et al., 2006). These results suggested that *p*-coumaric acid and caffeic acid were the main functional substances of EAL. There might be a better efficacy to improve *p*-coumaric acid and caffeic acid ratio in medicinal research and practice in clinic.

In our study, there might be a relationship between the above conversion and the enzymes present in the liver and small intestine. According to previous literature, many phenolic acids were the substrates of cytochrome P450 (CYP) (Martignoni et al., 2006). CYP1, CYP2, CYP3, and CYP4 families play a crucial role of the metabolism functions (Uno et al., 2008). The phenolic acids in EAL might become the substrates of CYPs during absorption because of the presence of CYPs in the small intestine and liver (Martignoni et al., 2004; Nakanishi et al., 2010). We found that the 1-O-caffeoylglycerol and 1-O-*p*-coumaroylglycerol levels in EAL decreased, and *p*-coumaric acid increased in the microsome incubations of liver and small intestine compared with that of normal saline. We may therefore conclude that the phenolic components were absorbed mainly in the form of *p*-coumaric acid and caffeic acid.

CYP subtypes present in liver and small intestine are different (Ramiro et al., 2009). For example, CYP1A2, CYP2U1, and CYP2V1 are found only in the liver; CYP1B1, CYP2R1, and CYP2W1 are found only in the small intestine, although other CYPs have similar tissue distributions, their expressions are more or less different (Nelson et al., 2004; Choudhary et al., 2005). The modulation of cytochrome P450-linked enzyme activity by caffeic acid revealed that it was the substrate for CYP1A2 which only presented in liver (Teel et al., 1998; Huang et al., 2009). It is suggested that certain CYP enzymes of metabolizing caffeic acid are presented in the liver, not in the small intestines. We therefore might draw a word that 1-O-*p*-coumaroylglycerol could convert into *p*-coumaric acid and 1-O-caffeoylglycerol could convert into caffeic acid when it was absorbed through the small intestine.

Conclusion

In the present study, we first proved that there existed biotransformation among phenolic acids when they pass through the intestines and were absorbed into blood when the EAL was orally administrated. *p*-Coumaric acid and caffeic acid are the major ingredients of EAL when

absorp orally. Since *A. comosus* leaves are the huge products all over the world, this study will be benefit for understanding its *in vivo* behavior and developing of its pharmacology of anti-hyperlipidemia and anti-hyperglycemia.

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ABBREVIATIONS

EAL, *Ananas comosus* leaves; **HPLC,** high performance liquid chromatography; **TP,** total protein.

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