Eriodictyol, not its glucuronide metabolites, attenuates acetaminophen-induced hepatotoxicity

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Figure 1. Intravenous eriodictyol administration decreases plasma transaminases, improves liver histology in mice with APAP-induced hepatotoxicity. (A–C) Plasma ALT, AST, and LDH levels. (D–H) H&E staining of liver sections, original magnification 200×: (D) control group, (E) eriodictyol 200 mg/kg (iv) group, (F) APAP 250 mg/kg group, (G) eriodictyol 50 mg/kg (iv)+APAP group, (H) eriodictyol 200 mg/kg (iv)+APAP group. The values presented are the mean ± S.D. (n=4–6/group), *P<0.05, **P<0.01, and ***P<0.001.

Figure 2. Intragastric eriodictyol administration fails to prevent APAP-induced hepatotoxicity. (A–E) H&E staining of the liver sections, original magnification 200×: (A) control group, (B) eriodictyol 200 mg/kg (ig) group, (C) APAP 250 mg/kg group, (D) eriodictyol 50 mg/kg (ig)+APAP group, (E) eriodictyol 200 mg/kg (ig)+APAP group. (F–H) Plasma ALT, AST, and LDH levels. The values presented are the mean ± S.D. (n=4–6/group), *P<0.05, **P<0.01, and ***P<0.001.

Figure 3. Intraperitoneal eriodictyol administration at higher concentration prevents APAP-induced hepatotoxicity. (A–E) H&E staining of the liver sections, original magnification 200×: (A) control group, (B) eriodictyol 200 mg/kg (ip) group, (C) APAP 250 mg/kg group, (D) eriodictyol 50 mg/kg (ip)+APAP group, (E) eriodictyol 200 mg/kg (ip)+APAP group. (F–H) Plasma ALT, AST, and LDH levels. The values presented are the mean ± S.D. (n=4–6/group), *P<0.05, **P<0.01, and ***P<0.001.

Figure 4. Two monoglucuronide metabolites of eriodictyol were identified by UPLC–ESI (−)–Q-TOF–MS. (A) Representative UPLC chromatograms for the quantitative analyses of eriodictyol and its metabolites in various liver microsomes. (B) MS/MS spectrum of eriodictyol metabolites. (C) Structure of eriodictyol and probable structure of the metabolites.

Figure 5. Glucuronidation of eriodictyol by 12 recombinant human UGT isoforms. Glucuronidation rates were calculated and expressed as nmol/min/mg protein. Each bar is the average of three determinations, and error bars are the standard deviations of the means.

Figure 6. Kinetic profiles for eriodictyol M1 glucuronidation by recombinant human UGT isoforms. In each panel, the insert shows the corresponding Eadie–Hofstee plot. Please refer to Table 1 for the incubation conditions of eriodictyol and Table 2 for derived kinetic parameters. Each point is the average of three determinations, and error bars are the standard deviations of the means.

Figure 7. Kinetic profiles for eriodictyol M2 glucuronidation by recombinant human UGT isoforms. In each panel, the insert shows the corresponding Eadie–Hofstee plot. Please refer to Table 1 for the incubation conditions of eriodictyol and Table 2 for the derived kinetic parameters. Each point is the average of three determinations, and error bars are the standard deviations of the means.

Figure 8. Comparisons of the intrinsic clearance (CLint) values for eriodictyol glucuronidation by recombinant human UGT isoforms and effects of chemical inhibitors on eriodictyol glucuronidation. Error bars are the standards deviations of three measurements. The symbol “−” indicates a comparison with all groups. The symbol “**” indicates a statistically significant difference.

Figure 9. Kinetic profiles for eriodictyol M1 glucuronidation by various liver and intestinal microsomes. In each panel, the insert shows the corresponding Eadie–Hofstee plot. Please refer to Table 1 for the incubation conditions of eriodictyol and Table 2 for the derived kinetic parameters. Each point is the average of three determinations, and error bars are the standard deviations of the means.
Figure 10. Kinetic profiles for eriodictyol M2 glucuronidation by various liver and intestinal microsomes. In each panel, the insert shows the corresponding Eadie–Hofstee plot. Please refer to Table 1 for the incubation conditions of eriodictyol and Table 2 for the derived kinetic parameters. Each point is the average of three determinations, and error bars are the standard deviations of the means.

Figure 11. Intestinal absorption and metabolism characteristics of eriodictyol in mice. Perfusate contained 20 μM eriodictyol. Small and colon intestinal segments were perfused at a flow rate of 10 mL/h. Data are expressed as mean ± S.D. (n=5/group). *P<0.05, **P<0.01, and ***P<0.001.

Figure 12. PK–PD analyses of intragastric and intraperitoneal eriodictyol administration at the early stage after APAP dosing. (A) Time course of plasma ALT level after APAP dosing. (B) Time course of plasma AST level after APAP dosing. (C) Pharmacokinetic distribution of eriodictyol in the plasma. (D) Pharmacokinetic distribution of metabolites in the plasma. Data are expressed as mean ± S.D. (n=4-6/group). *P<0.05, **P<0.01, and ***P<0.001.

Figure 13. The area under concentration–time curve of eriodictyol when combined with or without GA (an inhibitor of both UGT1As and UGT2B7). Mice were intragastrically administered with eriodictyol (40 mg/kg) with or without GA (300 mg/kg). Data are expressed as mean ± S.D. (n=6/group).

Figure 14. Intragastric eriodictyol administration combined with GA prevents APAP-induced hepatotoxicity. (A–D) H&E staining of the liver sections, original magnification 200×: (A) APAP 250 mg/kg group, (B) GA 300 mg/kg (ig) + APAP group, (C) Eriodictyol 200 mg/kg (ig) + APAP group, (D) GA 300 mg/kg (ig) + Eriodictyol 200 mg/kg (ig) + APAP group. (E and F) Plasma ALT and AST levels. (G–I) Plasma and liver concentrations of Eriodictyol and its metabolites. Data are expressed as mean ± S.D. (n=4-6/group). *P<0.05, **P<0.01, and ***P<0.001.

Figure 15. Intragastric eriodictyol administration prevents APAP-induced hepatotoxicity in Ugt1−/− mice but not in its wild-type littermates. (A–D) H&E staining of the liver sections, original magnification 200×: (E and F) Plasma ALT and AST levels. (G–H) Plasma and liver concentrations of eriodictyol and its metabolites. Data are expressed as mean ± S.D. (n=6/group). *P<0.05, **P<0.01, and ***P<0.001.

Figure 16. Intravenous eriodictyol administration decreases the activity of caspase-3 during APAP-induced hepatotoxicity. Data are expressed as mean ± S.D. (n=4-5/group). *P<0.05, **P<0.01, and ***P<0.001.

Figure 17. Intravenous eriodictyol administration reduces hepatocyte apoptosis during APAP-induced hepatotoxicity. TUNEL-staining analysis of paraffin-embed livers, original magnification, 100×. TUNEL-positive cells were stained in control group, eriodictyol 200 (iv) group, APAP group, eriodictyol 50 (iv) + APAP group and eriodictyol 200 (iv) + APAP group. DAPI stained all cells. FITC stained apoptotic cells. Merged was a superimposed picture.

Figure 18. Intravenous eriodictyol administration significantly inhibits the activities of cyp2e1 and cyp3a11 during APAP-induced hepatotoxicity. The values presented are the mean ± S.D. (n=4-5/group). *P<0.05, **P<0.01, and ***P<0.001.

Figure 19. Intravenous eriodictyol administration increases liver GSH content and the activities of GR, GSH-Px and GST during APAP-induced...
hepatotoxicity. The values presented are the mean ± S.D. (n=4-5/group). The prot is the abbreviation of protein *P<0.05, **P<0.01, and ***P<0.001.

**Figure 20.** Intravenous eriodictyol administration significantly decreases hepatic MDA level and increases the activity of SOD, with slightly reduction of plasma TNF-α and IL-1β levels during APAP-induced hepatotoxicity. The values presented are the mean ± S.D. (n=4-5/group). The prot is the abbreviation of protein *P<0.05, **P<0.01, and ***P<0.001.

**Table 1.** Incubation conditions for the glucuronidation assay of eriodictyol with various microsomes.

**Table 2.** Kinetic parameters derived for eriodictyol glucuronidation by various microsomes. Data are expressed as mean ± S.D. (n=3). SI stands for substrate inhibition model. MM stands for Michaelis–Menten model. NA stands for not available.

**Table 3.** Pharmacokinetic parameters after intragastric eriodictyol administration (40 mg/kg) with or without GA (300 mg/kg) in mice. Data are expressed as mean ± S.D. (n=6/group). *P<0.05, **P<0.01, and ***P<0.001.
Figure 1.

A  ALT

B  AST

C  LDH

D  Control

E  Eriodictyol 200 (iv)

F  APAP

G  Eriodictyol 50 (iv) + APAP

H  Eriodictyol 200 (iv) + APAP
Figure 2.

A Control | B Eriodictyol 200 (ig) | C APAP | D Eriodictyol 50 (ig) + APAP | E Eriodictyol 200 (ig) + APAP

F ALT

G AST

H LDH

***

IU/L

***

IU/L

***

U/L

ACS Paragon Plus Environment
Figure 3.

A Control
B Eriodictyol 200 (ip)
C APAP
D Eriodictyol 50 (ip) + APAP
E Eriodictyol 200 (ip) + APAP

F

ALT

IU/L

**

G

AST

IU/L

**

H

LDH

U/L

***

ACS Paragon Plus Environment
Figure 5.
Figure 6.

A UGT1A1

V (nmol/min/mg)

0.0  0.4  0.8  1.2  1.6

Con (μM)

0  200  400  600  800

V/C

B UGT1A3

V (nmol/min/mg)

0.0  0.4  0.8  1.2  1.6

Con (μM)

0  50  150  200

V/C

C UGT1A6

V (nmol/min/mg)

0.0  0.6  1.2  1.8  2.4

Con (μM)

0  200  400  600  800

V/C

D UGT1A7

V (nmol/min/mg)

0.0  0.6  1.2  1.8  2.4

Con (μM)

0  10  20  30  40  50

V/C

E UGT1A8

V (nmol/min/mg)

0.0  0.3  0.6  0.9  1.2

Con (μM)

0  100  200  300  400

V/C

F UGT1A9

V (nmol/min/mg)

0.0  0.3  0.6  0.9  1.2

Con (μM)

0  100  200  300  400

V/C

G UGT1A10

V (nmol/min/mg)

0.00  0.05  0.10  0.15  0.20

Con (μM)

0  50  100  150  200

V/C

H UGT2B7

V (nmol/min/mg)

0.00  0.05  0.10  0.15  0.20

Con (μM)

0  100  200  300

V/C

I UGT2B15

V (nmol/min/mg)

0.00  0.05  0.10  0.15  0.20

Con (μM)

0  100  200  300

V/C

J UGT2B17

V (nmol/min/mg)

0.00  0.05  0.10  0.15  0.20

Con (μM)

0  100  200  300

V/C
Figure 7.
Figure 8.
Figure 9.
Figure 10.
Figure 11.

A

Amount of Eriodictyol Absorbed (nmol/30min)

0 8 16 24 32

Small Intestine Colon

***

B

Amount of Eriodictyol Metabolized (nmol/30min)

0.0 0.8 1.6 2.4 3.2

Small Intestine Colon

M1 M2

***
Figure 12.

A. ALT

B. AST

C. Plasma Eriodictyol (μM)

D. Plasma Metabolites (μM)
Figure 13.
Figure 14.

A APAP

B GA 300 (ig) + APAP

C Eriodictyol 200 (ig) + APAP

D GA + Eriodictyol + APAP

E

ALT

IU/L

Control
Eriodictyol 200 (ig)
APAP
GA 300 (ig) + APAP
Eriodictyol 200 (ig) + APAP

0
75
150
225
300

***

* *

F

AST

IU/L

Control
Eriodictyol 200 (ig)
APAP
GA 300 (ig) + APAP
Eriodictyol 200 (ig) + APAP

0
50
100
150
200

***

* *

G

Plasma Eriodictyol (μM)

24h

0.00
0.06
0.12
0.18
0.24

Eriodictyol + APAP
GA + Eriodictyol + APAP

H

Liver Eriodictyol (μM)

24h

0.0
0.5
1.0
1.5
2.0

Eriodictyol + APAP
GA + Eriodictyol + APAP

I

Plasma Metabolites (μM)

24h

0.00
0.05
0.10
0.15
0.20

Eriodictyol + APAP
GA + Eriodictyol + APAP

*
Figure 15.

A Wild-type APAP

B Wild-type APAP+Eriodictyol

C Ugt1<sup>+/−</sup> APAP

D Ugt1<sup>+/−</sup> APAP+Eriodictyol

E ALT

F AST

G Plasma Eriodictyol (μM)

H Liver Eriodictyol (μM)

I Plasma Metabolites (μM)
Figure 16.

Caspase-3 activity (fold)

- Control
- Eriodictyol 200 (iv)
- APAP
- Eriodictyol 50 (iv) + APAP
- Eriodictyol 200 (iv) + APAP
**Figure 17.**

Control

- DAPI (Blue)
- FITC (Green)
- Merged

Eriodictyol 200 (iv)

- DAPI (Blue)
- FITC (Green)
- Merged

APAP

- DAPI (Blue)
- FITC (Green)
- Merged

Eriodictyol 50 (iv) + APAP

- DAPI (Blue)
- FITC (Green)
- Merged

Eriodictyol 200 (iv) + APAP

- DAPI (Blue)
- FITC (Green)
- Merged
Figure 18.

A

B

cyp2e1 activity (percent of Control group)

0 40 80 120 160

Control Eriodictyol 200 (iv) APAP Eriodictyol 50 (iv) +APAP Eriodictyol 200 (iv) +APAP

***
cyp3a11 activity (percent of Control group)

0 75 150 225 300

Control Eriodictyol 200 (iv) APAP Eriodictyol 50 (iv) +APAP Eriodictyol 200 (iv) +APAP

* **
Figure 19.
### Figure 20.

#### Liver MDA (nmol/mgprot)

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#### Liver SOD (U/mgprot)

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#### TNF-α (pg/mL)

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#### IL-1β (pg/mL)

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<td>2.32 ± 0.59 **</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>min</td>
<td>77.92 ± 27.31</td>
<td>86.91 ± 27.18</td>
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<tr>
<td>$AUC_{0-t}$</td>
<td>μmol/L*min</td>
<td>26.90 ± 5.30</td>
<td>58.33 ± 9.76 *</td>
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<tr>
<td>$AUC_{0-inf}$</td>
<td>μmol/L*min</td>
<td>37.98 ± 9.56</td>
<td>111.17 ± 49.12 ***</td>
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<tr>
<td>$MRT$</td>
<td>min</td>
<td>112.44 ± 27.13</td>
<td>125.41 ± 39.22</td>
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<tr>
<td>$F_{rel}$ (%)</td>
<td></td>
<td>216.84 %</td>
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Eriodictyol, not its glucuronide metabolites, attenuates acetaminophen-induced hepatotoxicity

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Abstract

Acetaminophen (APAP) is one of the most commonly used oral analgesics and antipyretics, but hepatotoxicity including liver failure may occur after overdose. The therapeutic options for treating APAP hepatotoxicity are limited. Eriodictyol, a dietary flavonoid with anti-inflammatory and antioxidant properties, was used here to determine its protective effects against APAP-induced hepatotoxicity in mice. Various administration routes and pharmacokinetics–pharmacodynamics (PK–PD) analyses were used to determine these effects. Protective effects were observed in intravenously and intraperitoneally but not in intragastrically administered eriodictyol. LC-MS/MS analysis revealed two monoglucuronide metabolites of eriodictyol in liver and intestine microsomes. Recombinant human uridine-5’-diphospho-glucuronosyltransferase (UGT) isoforms and chemical inhibition studies demonstrated that UGT1As (mainly UGT1A1, UGT1A9, UGT1A10) and UGT2B7 were likely the main contributors to eriodictyol glucuronidation. Intragastric administration of eriodictyol, which displayed lower parent and higher metabolite concentrations in the plasma, did not elicit protective effects against APAP hepatotoxicity, when compared to the intraperitoneal injection of eriodictyol. The relative bioavailability of eriodictyol was increased to 216.84% with the co-administration of glycyrrhetic acid (GA), an inhibitor of UGT1As. Intragastric administration of eriodictyol in combination with GA also induced protective effects against APAP hepatotoxicity. Furthermore, intragastric administration of eriodictyol attenuated APAP hepatotoxicity in Ugt1+/− mice but not in its wild-type littermates. Thus, UGT1A-mediated metabolic inactivation reduced the protective effect of eriodictyol. Eriodictyol attenuated APAP hepatotoxicity via inhibition of hepatic cytochrome P450 (cyp) 2e1 and cyp3a11 activities, reserve of glutathione (GSH) by improvement of glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione S-transferase (GST) activities, elevation of superoxide dismutase (SOD) activity, and reduction of malondialdehyde (MDA) level. Our findings indicate that parenterally administered eriodictyol may be used to treat APAP-induced hepatotoxicity, and its efficacy can be enhanced by UGT1As down-regulation.

Keywords: Eriodictyol; glucuronidation; Ugt1+/− mice; acetaminophen; hepatotoxicity.
**Introduction**

Acetaminophen (N-acetyl-p-aminophenol, APAP), a widespread and effective analgesic and antipyretic drug, can cause severe liver injury or even acute liver failure when it is used with acute or cumulative overdose. In the United States, around 30,000 people every year are subjected to APAP hepatotoxicity. And clinical data has shown that 29% of patients with acute liver failure secondary to APAP toxicity undergo liver transplant. APAP overdose produces an excessive toxic intermediate, namely, N-acetyl-p-benzoquinone imine (NAPQI), through the metabolism mediated via cytochromes P450 (CYP) enzymes, including cyp2e1, cyp3a11, which reacts rapidly with glutathione (GSH). GSH depletion consequently exacerbates oxidative stress, causes mitochondrial dysfunction, impairs liver functions, and ultimately causes hepatocyte necrosis/apoptosis. Currently, the clinical choice for APAP overdose treatment is N-acetyl-cysteine (NAC). NAC protects against APAP hepatotoxicity by enhancing GSH stores and increasing nontoxic sulfate conjugation. Both oral and intravenous NAC are effective in preventing liver injury, although side effects of NAC including nausea, vomiting, diarrhea or constipation are not ignorable. Additionally, NAC is ineffective in some instances, because it needs to be administered very early prior to NAPQI binding to cellular proteins. Therefore, new antidotes are urgent need. According to the toxic mechanisms of APAP, any agent with the ability to inhibit cyp2e1 and cyp3a11 activities, reserve GSH, and/or attenuate oxidative and inflammatory stress may potentially attenuate APAP related liver injury.

Flavonoids, present in vegetables and fruits and possessed beneficial biological activities. Eriodictyol [2-(3, 4-dihydroxyphenyl)-5, 7-dihydroxy-2, 3-dihydrochromen-4-one] is an interesting flavonoid commonly present in vegetables and fruits especially citrus such as lemon and has anti-inflammatory and antioxidant properties. Previous studies showed that eriodictyol could alleviate the LPS-induced lung injury in mice by regulating the Nrf2 pathway and inhibiting the expression of inflammatory cytokines in macrophages. Eriodictyol also protects endothelial cells against oxidative stress-induced cell death. Eriodictyol has been reported to prevent cisplatin-induced kidney injury by activating Nrf2 and inhibiting NF-κB activation. Furthermore, it has been demonstrated that eriodictyol exhibit promising neuroprotection effects against the...
permanent focal ischemia cerebral injury in the mice through inhibition of neuroinflammation\textsuperscript{20}. Preliminary results of our research group found that eriodictyol could inhibit the activity of cyp2e1 which mediates the formation of NAPQI, the toxic metabolite of APAP. In the light of these study results, we hypothesis that eriodictyol could alleviate APAP-induced hepatotoxicity. Therefore, the purpose of this study was to determine the hepatoprotective effects of eriodictyol and its underlying mechanisms.

As is known to all, flavonoids are extensively subjected to phase II metabolism, especially glucuronidation. Glucuronidation is a typical phase II metabolic reaction mediated by uridine-5’-diphospho-glucuronosyltransferase (UGTs). In humans, functional UGT isoforms are classified into four families, namely, UGT1, UGT2, UGT3 and UGT8. UGT1A, UGT2B and UGT3 families participate in drug metabolism, whereas UGT8 performs biosynthetic function in the nervous system\textsuperscript{21,22}. UGT1A and UGT2B play a dominant role in the disposition of flavonoids, such as genistein\textsuperscript{23}, daidzein\textsuperscript{24}, chrysin\textsuperscript{25}, quercetin\textsuperscript{26}, and luteolin\textsuperscript{27}. Eriodictyol has very similar chemical structure of luteolin. Consequently, eriodictyol is probably metabolized by UGTs.

In present study, we will also identify the active substance that elicits protective effects of eriodictyol through pharmacokinetics-pharmacodynamics (PK–PD) analyses. Ugt1\textsuperscript{+/-} mice and its wild-type littermates were subjected to histological and biochemical analyses. Chemical inhibition and recombinant enzyme experiments were conducted to characterize the underlying metabolic mechanisms. This study is the first to investigate the active substance and mechanism for the protective effect of eriodictyol against APAP-induced hepatotoxicity. Our results can be used as a basis for further studies on eriodictyol.

**Materials and methods**

**Materials**

Eriodictyol, glycyrrhetic acid (GA), atractylenolide I, atazanavir, and genistein (purity >98%) were all purchased from Sichuan Victory Biotechnology Co., Ltd. (Sichuan, China). APAP was
bought from Aladdin Shanghai Biochemical Technology Co., Ltd. (Shanghai, China). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD), malondialdehyde (MDA) and Caspase-3 kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Enzyme-linked immunosorbent assay kits for mouse TNF-α and IL-1β were purchased from NeoBioscience Biotechnology Co., Ltd (Shenzhen, China). Recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17) and pooled human liver microsomes (HLMs) and human intestine microsomes (HIMs) were obtained from BD Biosciences (Woburn, MA, USA). Pooled mice liver microsomes (MLMs) and mice intestine microsomes (MIMs) were prepared based on the methods used in previous studies in our lab. Uridine diphosphoglucuronic acid (UDPGA), alamethicin and D-saccharic-1, 4-lactone monohydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials (typically analytical grade or better) were used as received.

**Animals**

Kunming (KM) mice (male, 18–22 g) were obtained from the Experimental Animal Center of Southern Medical University (Guangzhou, China). Ugt1+/− mice and its wild-type mice with a C57BL/6N genetic background (18-22 g) were provided by the International Institute for Translational Chinese Medicine, Guangzhou University of Chinese Medicine (Guangzhou, China). The animal room was maintained at 23±1 °C with a 12 h light–dark cycle and 55 ± 5% humidity. The animal studies were approved by the Animal Ethics Committee of Southern Medical University and Guangzhou University of Chinese Medicine (Guangzhou, China).

**Experimental design**

**Evaluation of the protective effect of eriodictyol on APAP-induced hepatotoxicity through various administration routes**

KM mice were fasted overnight with free access to water before intraperitoneal dosing of APAP. Mice were administered 250 mg/kg of APAP to induce hepatotoxicity. Intragastric administration
or intraperitoneal injection of eriodictyol (50 or 200 mg/kg) was performed at 30 min before APAP dosing. Intravenous injection of eriodictyol (50 or 200 mg/kg) was co-administered with APAP. Blood and livers were collected at 24 h after APAP dosing. For PK-PD analyses, 200 mg/kg of eriodictyol was intragastrically administered or intraperitoneally injected to mice, and the mice were killed at 1, 2, 4, 6, 8, and 24 h after APAP dosing, and then blood and livers were collected and analyzed. A portion of the liver was fixed in 10% formalin solution, and the remaining liver was flash frozen in liquid nitrogen and stored at −80 °C for further use. Formalin-fixed liver tissues were embedded in paraffin, and 4 µm-thick sections were cut for hematoxylin and eosin (H&E) staining according to the manual protocols. The levels of ALT, AST and LDH in plasma were quantified using a commercial assay kit based on the manufacturer’s instructions. For quantitative analysis of eriodictyol and its metabolites by ultraperformance liquid chromatography (UPLC)–mass spectrometry (MS)/MS, plasma or liver supernatant was spiked with acetonitrile containing genistein (internal standard, IS), and then vortexed for 3 min. After centrifugation at 13000 rpm for 30 min, the supernatant was transferred to a new tube and evaporated to dryness in a vacuum drying oven. The residue was reconstituted with 30% acetonitrile aqueous solution and centrifuged at 13,000 rpm for 30 min before injection.

**Evaluation of the metabolic mechanism of eriodictyol in vitro**

In glucuronidation reaction\(^{29}\), the incubation mixture contained microsomes (13.25–53.00 µg protein/mL), magnesium chloride (0.88 mM), saccharolactone (4.4 mM), alamethicin (0.022 mg/mL), substrates with different concentrations, UDPGA (3.5 mM), and potassium phosphate buffer (50 mM, pH 7.4). The mixture was incubated at 37 °C for 30 min or 60 min, terminated with the addition of cold precipitating agent (94% acetonitrile, 6% acetic acid), vortexed for 3 min, and then centrifugation at 13,000 rpm for 30 min. The supernatant was subjected to analysis by UPLC. All incubations were performed in triplicate.

The metabolism of eriodictyol was measured using a panel of recombinant human UGT isoforms (including UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17) to identify the major UGT isoenzyme (s) involved
in the biotransformation of eriodictyol. The catalytic activities of individual UGT isoforms for eriodictyol were performed at 37 °C for 60 min with a final protein concentration of 53.00 µg protein/mL. All incubations were performed in triplicate.

Kinetic evaluations of eriodictyol glucuronidation were performed in various microsomes and individual UGT enzymes. Preliminary experiments were performed to ensure that the glucuronidation rates were determined under linear conditions with selected incubation time and protein concentrations. Table 1 lists the incubation conditions of the glucuronidation assay of eriodictyol. The kinetic parameters were obtained by fitting a one-enzyme Michaelis–Menten equation ($V = V_{max} \times S / (K_m + S + S/K_α)$), or Hill equation ($V = V_{max} \times S^n / (S_{50}^n + S^n)$). The kinetic parameters included the maximal velocity ($V_{max}$), substrate concentration at half-maximal rate ($K_m$ or $S_{50}$), substrate inhibition constant ($K_α$), intrinsic clearance ($CL_{int} = V_{max} / K_m$ or $S_{50}$), and Hill coefficient ($n$). Model fitting and parameter estimation were performed using the GraphPad Prism 5.0 software (San Diego, CA, USA). The apparent kinetic parameters were described as mean ± S.D. of triplicate samples.

Chemical inhibition assays were performed to further reveal the role of primarily involved UGT isoform in the hepatic eriodictyol glucuronidation. Atazanavir is an inhibitor of UGT1A1, UGT1A3 and UGT1A9, and atractylenolide I is a selective inhibitor of UGT2B7. A total of 400 µM eriodictyol (close to $K_m$ of eriodictyol’s metabolite 1 [M1]) was incubated with HLMs (53.00 µg protein/mL) with or without atazanavir (19.2 µM). Furthermore, 100 µM eriodictyol (close to $K_m$ of eriodictyol’s metabolite 2 [M2]) was incubated with HLMs (53.00 µg protein/mL) with or without atractylenolide I (25.6 µM). All incubations were performed in triplicate.

The identification and determination of eriodictyol and its metabolites were conducted under the following analytical UPLC conditions: systems, Agilent 1290 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA); column, Agilent Poroshell 120 SB-C18 (50×2.1 mm, 2.7 µm; Agilent Technologies, Santa Clara, CA, USA); mobile phase B, 100% acetonitrile; mobile phase A, 0.1% formic acid in water; flow rate, 0.35 mL/min; gradient elution, 0–1.5 min for 98% A, 1.5–2.0 min for 98%–80% A, 2.0–4.5 min for 80%–50% A, 4.5–5 min for 50%–10% A, and
5–7 min for 10%–98% A; wavelength, 330 nm; and injection volume, 10 µL. The structural identification of eriodictyol glucuronides was performed using Agilent 6540 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) MS System (Agilent Technologies, Santa Clara, CA, USA). The mass spectrometer was operated in negative ion electrospray ionization mode for the determination. The main working parameters were set as follows: capillary voltage, 2.5 KV; cone voltage, 25 V; drying gas temperature, 300 °C; and sheath gas temperature, 250 °C. Nitrogen was used as the sheath and drying gas at flow rates of 11.0 and 5.1 L/min, respectively. Data acquisition and analysis were performed using Agilent MassHunter version B.06.00 software (Agilent Technologies, Santa Clara, CA, USA). Given the lack of reference standard, the quantification of eriodictyol glucuronide was based on the standard curve of the parent compound (eriodictyol) and further calibrated using the correction factors.

**Evaluation of eriodictyol metabolism in situ in mice**

KM mice were fasted overnight with free access to water before the experiments. The intestinal surgical procedures were modified based on previously described techniques. After the surgery, small intestinal and colon intestinal segments were simultaneously cannulated with a perfusate containing 20 µM eriodictyol at a flow rate of 10 mL/h. After a 30 min washout period, perfusate samples from perfused intestinal segments were collected every 30 min. After perfusion, the length of each intestinal segment was measured, and each tube containing the sample was weighed as previously described. The perfusate samples were added equal volume of methanol containing 10 µM IS. After centrifugation at 13,000 rpm for 30 min, the supernatant was injected to UPLC for determination of the concentration of eriodictyol and its metabolites.

**Effect of GA on the pharmacokinetic behavior of eriodictyol in vivo**

GA is an inhibitor of UGT1As and UGT2B7. KM mice were randomly divided into two groups (n=6 each). In the eriodictyol alone group, mice were administered with a single dose of eriodictyol (40 mg/kg) by intragastric administration. In the eriodictyol/GA group, the mice were administered with a single dose of combined GA (300 mg/kg)) and eriodictyol (40 mg/kg). The blood was collected from the orbital venous at 0, 2, 5, 10, 15, 25, 60, 120 and 240 min after dosing.
The plasma samples were prepared as mentioned above. The concentration of eriodictyol in the plasma was detected by UPLC–MS/MS. The pharmacokinetic parameters of eriodictyol were calculated using the Drug and Statistics for Windows software package (version 2.1, Chinese Pharmacological Society).

**Effect of GA on the protective effect of eriodictyol on APAP-induced hepatotoxicity**

Mice were intragastrically administered with eriodictyol (200 mg/kg) in the presence or absence of GA (300 mg/kg) at 30 min before APAP dosing (250 mg/kg). The effect of GA alone (300 mg/kg) on APAP hepatotoxicity was also detected. The blood and livers were collected at 24 h after APAP dosing.

**Evaluation of the protective effect of eriodictyol by intragastric administration on APAP-induced hepatotoxicity in Ugt1<sup>+/−</sup> mice and its wild-type mice**

Ugt1<sup>+/−</sup> mice and its wild-type mice were given eriodictyol (200 mg/kg) by intragastric administration at 30 min before APAP dosing (300 mg/kg). The blood and livers were collected at 24 h after APAP dosing.

**Investigation on the mechanism of eriodictyol alleviating APAP-induced hepatotoxicity**

KM Mice were administered 250 mg/kg of APAP to induce hepatotoxicity. Intravenous injection of eriodictyol (50 or 200 mg/kg) was co-administered with APAP. Blood and livers were collected at 24 h after APAP dosing. Formalin-fixed liver tissues were embedded in paraffin for terminal deoxynucleotidyl transferase–mediated digoxigenin-deoxyuridine nick-end labeling (TUNEL) staining according to the manual protocols. The remaining liver tissues were homogenized with saline and centrifuged at 3500 rpm for 10 min at 4 °C, and then the supernatant was collected for detection of Caspase-3, GSH, GSH-PX, GR, GST, SOD and MDA by commercial assay kits based on the manufacturer’s instructions. The levels TNF-α, and IL-1β in plasma were also quantified using a commercial assay kits based on the manufacturer’s instructions. The activities of cyp2e1 and cyp3a11 in the liver were detected using probe substrates (chlorzoxazone for cyp2e1, testosterone for cyp3a11) incubated with liver microsomes of each group.
Statistical analysis

One-way analysis of variance with or without Tukey–Kramer multiple comparison (posthoc) tests were used to evaluate statistical differences. Differences were considered significant when \( P \)-values were less than 0.05.

Results

**Intravenous administration of eriodictyol reduces APAP-induced hepatotoxicity**

Plasma ALT, AST and LDH were measured as biochemical makers to evaluate the extent of liver injury and to determine the effects of intravenous eriodictyol administration on APAP-induced hepatotoxicity. As expected, the APAP-treated group sharply increased plasma ALT, AST and LDH when compared to control group, whereas eriodictyol/APAP-treated group displayed markedly decreased levels of ALT, AST and LDH when compared to APAP-treated group (Figure 1A–C). Histological analysis demonstrated that livers in the control (Figure 1D) and eriodictyol (Figure 1E) groups displayed normal lobular architecture and cell structure. APAP treatment caused severe hepatocellular necrosis (Figure 1F). Nevertheless, necrotic areas of the liver were markedly decreased in the eriodictyol/APAP-treated group (Figure 1G–H).

**Intragastric administration of eriodictyol did not affect APAP-induced hepatotoxicity**

A single dose of eriodictyol at two concentrations administered by intragastric administration showed no effects on the attenuation of APAP-induced hepatotoxicity. For eriodictyol/APAP-treated group, necrotic areas of the liver did not significantly decrease after APAP poisoning (Figure 2A–E). Additionally, plasma ALT, AST and LDH were significantly higher than the control group, although no significant differences was detected when compared with the APAP alone group (Figure 2F–H). Intraperitoneal eriodictyol injection at high concentration (200 mg/kg) significantly decreased the APAP-induced hepatocyte death and markedly attenuated the increased plasma ALT, AST and LDH, although these effects were not observed at low concentration (Figure 3A–H).

**Two monoglucuronide metabolites of eriodictyol are found in liver microsomes**

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Two eriodictyol metabolites (M1 and M2) were observed after incubation with HLMs and MLMs in the presence of UDPGA. The retention time of M1, M2 and eriodictyol in the UPLC chromatography were 3.3, 3.6 and 4.1 min, respectively (Figure 4A). The structural identification by high-resolution mass spectrometry (HRMS) showed that the two metabolites were both identified as monoglucuronides of eriodictyol. The molecular formula of the two metabolites was C_{21}H_{19}O_{12} \ (m/z \ 463.0880), which indicated that a C_6H_8O_6 (m/z 176) moiety was added by comparing with the C_{15}H_{12}O_6 (m/z 287.0564), the molecular formula of eriodictyol (Figure 4B). This finding suggested that UGT transferred a glucuronyl group to eriodictyol. Hence, two monoglucuronide metabolites of eriodictyol were generated by UGTs in the liver.

**UGT1As and UGT2B7 predominantly mediate eriodictyol glucuronidation in vitro**

Twelve expressed human UGTs (including UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17) were used to catalyze the eriodictyol glucuronidation and identify dominant UGT isoforms involved in eriodictyol glucuronidation. The results demonstrated that 10 isoforms, namely, UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15 and UGT2B17, were involved in M1 formation. Except UGT1A4, UGT1A6, UGT1A7, UGT2B4 and UGT2B17, the seven remaining isoforms showed metabolic activities toward M2 formation (Figure 5). The kinetic analyses of the eriodictyol glucuronide formation were performed with UGT isoforms that mediated eriodictyol metabolism (Table 1). The M1 formation mediated by UGT1A1, UGT1A8, UGT1A10 and UGT2B15 was best fitted by substrate inhibition equation, whereas that mediated by UGT1A3, UGT1A6, UGT1A7, UGT1A9, UGT2B7 and UGT2B17 was best fitted by Michaelis–Menten equation (Figure 6). The M2 formation mediated by UGT1A1, UGT1A10, UGT2B7 and UGT2B15 was best fitted by substrate inhibition equation, whereas that mediated by UGT1A3, UGT1A8 and UGT1A9 was best fitted by Michaelis–Menten equation (Figure 7). Table 2 displays the kinetic parameters estimated from recombinant UGT isoforms. Based on the intrinsic clearance (CL_{int}) values, the rank order for the eriodictyol glucuronidation was UGT1A1 (290.24±11.27 µl/min/mg) > UGT2B7 (234.94±3.15 µl/min/mg) > UGT1A10 > UGT1A9 > UGT1A3 > UGT2B17 (35.53±3.27 µl/min/mg) (Figure 8A).
Eriodictyol glucuronidation by liver and intestinal microsomes was determined at different substrate concentrations. Table 1 lists the conditions for kinetic analyses of eriodictyol glucuronidation. Table 2 presents the kinetic parameters estimated from different microsomes. Both M1 and M2 formation in HLMs and HIMs were best fitted by substrate inhibition equation, and those in MLMs and MIMs were best fitted by Michaelis–Menten equation and substrate inhibition equation, respectively (Figure 9–10).

An in situ mice single-pass intestinal perfusion model was used to further confirm the role intestinal enzymes in eriodictyol glucuronidation. The amount of eriodictyol absorption in small intestine was significantly higher than that in colon, with the value of 25.27±1.84 and 1.98±0.79 nmol, respectively. The amount of M1 generated in small intestine was 1.76 ± 0.47 nmol, and that of M2 was 0.38±0.09 nmol. No monoglucuronide metabolites were found in the colon (Figure 11). Therefore, the intestine was also a metabolic organ where eriodictyol glucuronidation occurred.

**PK–PD studies of eriodictyol on APAP-induced hepatotoxicity**

PK–PD analyses of eriodictyol (200 mg/kg) administered intragastrically or via intraperitoneal injection before the APAP challenge were performed. The results indicated that plasma ALT and AST in the intragastric administration group were significantly higher than those in the intraperitoneal injection group at 8 h and 24 h after APAP challenge (P<0.05) (Figure 12A–B). Notably, eriodictyol concentration in the intragastric administration group was significantly lower than that in intraperitoneal injection group at different time points, but the metabolites displayed an opposite trend (Figure 12C–D). These data preliminarily suggested that eriodictyol, not its monoglucuronide metabolites, attenuated APAP hepatotoxicity.

**GA, an inhibitor of UGT1As, increases the bioavailability of eriodictyol in vivo**

Pharmacokinetic behaviors of eriodictyol in the presence or absence of GA were investigated. Figure 13 exhibits the plasma eriodictyol concentration–time profiles. Table 3 summarizes the pharmacokinetic parameters of eriodictyol. The $C_{max}$ value of eriodictyol was 1.36±0.11 µmol/L, which significantly increased to 2.32±0.59 µmol/L in the presence of GA (P<0.001). Eriodictyol
exposure in presence of GA, with a 2.17-fold increase in AUC value \((P<0.05)\), was significantly greater than that of eriodictyol alone. The relative bioavailability of eriodictyol in the presence of GA was calculated as 216.84%. Thus, GA, an inhibitor of UGT1As, increased the bioavailability of eriodictyol in vivo.

**GA enhances the protective effect of eriodictyol on APAP hepatotoxicity**

Plasma ALT and AST were significantly reduced in intragastric GA/eriodictyol/APAP-treated group \((P<0.05)\), which was not observed in GA or eriodictyol alone/APAP-treated group when compared to APAP-treated group (Figure 14E–F). Necrotic areas of the liver were also markedly decreased in GA/eriodictyol/APAP-treated group (Figure 14A–D). Eriodictyol concentrations of plasma and liver in the GA/eriodictyol/APAP-treated group were both significantly higher than those in the eriodictyol/APAP-treated group (Figure 14G–H). Conversely, plasma eriodictyol metabolites concentration in the GA/eriodictyol/APAP-treated group was significantly lower than that in the eriodictyol/APAP-treated group (Figure 14I). These results further indicated that eriodictyol, not its monoglucuronide metabolites, attenuated APAP hepatotoxicity.

**Intragastric administration of eriodictyol significantly reduces APAP-induced hepatotoxicity in Ugt1+/− mice but not in its wild-type littermates**

The protective effect of intragastric eriodictyol administration against APAP-induced hepatotoxicity in Ugt1+/− mice and its wild-type littermates were determined. As expected, a markedly decreased necrotic area of the liver was observed in Ugt1+/− mice after eriodictyol/APAP treatment, whereas this phenomenon was not observed in its wild-type littermates (Figure 15A-D). Furthermore, plasma ALT and AST were sharply decreased in the Ugt1+/− mice \((P<0.05)\) (Figure 15E-F). Eriodictyol concentrations of plasma and liver in the Ugt1+/− mice were both significantly higher than those in its wild-type littermates (Figure 15G–H). Conversely, plasma eriodictyol metabolites concentration in Ugt1+/− mice was significantly lower than that in its wild-type littermates (Figure 15I). These results demonstrated that eriodictyol, not its monoglucuronide metabolites, attenuated APAP hepatotoxicity.
**Eriodictyol attenuates apoptosis during APAP-induced hepatotoxicity**

The apoptosis of liver was assessed by using TUNEL staining and caspase-3 activity assay. The activity of caspase-3, a key downstream effector in apoptosis cascades, was significantly higher in the APAP-treated group when compared to the control group, whereas decreased in the eriodictyol/APAP-treated group (Figure 16). Significant green fluorescent staining of apoptotic cells was observed in the APAP-treated group. And less apoptotic cells were seen in the eriodictyol/APAP-treated group when compared to the APAP group (Figure 17). These results indicated that eriodictyol attenuated apoptosis during APAP-induced hepatotoxicity.

**Eriodictyol reduces hepatic cyp2e1 and cyp3a11 activities during APAP-induced hepatotoxicity**

The results shown that the activity of cyp2e1 was markedly decreased in eriodictyol (200 mg/kg)/APAP-treated group when compared to APAP-treated group, although eriodictyol alone didn’t decrease cyp2e1 activity (Figure 18A). The activity of cyp3a11 was also significantly decreased in eriodictyol (50 and 200 mg/kg)/APAP-treated group when compared to the APAP-treated group, although eriodictyol alone didn’t decrease cyp3a11 activity (Figure 18B). These results indicated that eriodictyol significantly inhibited activities of cyp2e1 and cyp3a11 during APAP-induced hepatotoxicity.

**Eriodictyol increases hepatic GSH content and GSH metabolic enzyme activities**

The depletion of GSH causes increased oxidative stress in the cells, and oxidative stress is the primary factor in APAP-induced hepatotoxicity. Liver GSH content in the APAP group was significantly lower when compared to the control group, but it significantly increased in the eriodictyol/APAP-treated group as well as eriodictyol-treated group (Figure 19A). Activities of hepatic GSH-Px, GR and GST, three hepatic GSH metabolic enzymes, were also increased in the eriodictyol/APAP-treated group when compared to the APAP-treated group (Figure 19B-D). These results indicated that eriodictyol stored GSH by improvement of GSH-Px, GR and GST activities during APAP-induced hepatotoxicity.

**Eriodictyol alleviates oxidative stress during APAP-induced hepatotoxicity**
Plasma TNF-α and IL-1β were slightly reduced in presence of eriodictyol in APAP-treated group (Figure 20A–B). MDA and SOD as biochemical makers to evaluate the antioxidant effect of eriodictyol were also measured. MDA was markedly decreased in the eriodictyol/APAP-treated group when compared to the APAP-treated group (Figure 20C). And the activity of SOD was significantly increased in the eriodictyol/APAP-treated group when compared to the APAP-treated group (Figure 20D). Thus, these results suggested that eriodictyol alleviated oxidative stress during APAP-induced hepatotoxicity.

**Discussion**

APAP is a widely used and effective antipyretic and analgesic drug. Unfortunately, APAP overdose is the leading cause of acute liver injury or even liver failure. Thus, the treatment for APAP-induced hepatotoxicity is highly desired. Eriodictyol, a flavonoid commonly distributed in vegetables and fruits, shows beneficial biological activities, such as antioxidant and anti-inflammatory actions, which are thought to be related to the inhibition of proinflammatory mediator release and reduction of oxidative stress. Nevertheless, the protective effects of eriodictyol against APAP-induced hepatotoxicity have not been reported. Meantime, the material basis of the pharmacological effects of a drug should be examined to promote its development. In the present study, we demonstrated that eriodictyol, not its monoglucuronide metabolites, attenuated APAP hepatotoxicity. Down-regulation of UGT1As enhanced the protective effect of eriodictyol. Eriodictyol attenuated APAP hepatotoxicity via inhibition of cyp2e1 and cyp3a11 activities, reserve of GSH by improvement of GSH-Px, GR and GST activities, elevation of SOD activity, and reduction of MDA level.

In the experiments, the role of eriodictyol on APAP hepatotoxicity was investigated through various administration routes. Unexpectedly, intravenous eriodictyol injection but not intragastric administration exerted a significantly protective effect through the marked reduction of necrosis (Figures 1–3). Further, we examined the metabolic mechanism of eriodictyol because numerous flavonoids have been reported to be extensively metabolized by UGTs. Flavonoids, which are a type of polyphenolic compound, mostly undergo UGT-mediated O-glucuronidation. UGTs
located at the endoplasmic reticulum can transfer the glucuronyl group from uridine-5’-diphosphoglucuronic acid to its substrate.\textsuperscript{41} Eriodictyol is possibly metabolized by UGTs because of its chemical structure that contains four OH groups. As expected, we found two eriodictyol monoglucuronide metabolites identified by HRMS in HLMs and MLMs (Figure 4). Our previous laboratory results revealed that the 7, 3’, and 4’ C-positions can all be associated with the glucuronyl group.\textsuperscript{42} Therefore, we did not conclude the exact position of glucuronidation of eriodictyol through HRMS. Recombinant human UGT isoforms and chemical inhibition experiments demonstrated that these metabolites were mainly metabolized by UGT1A\textsubscript{1} and UGT2B7 (Figures 5–8). In addition to liver, intestine is the main metabolic organ. Subsequently, we tested the metabolism of eriodictyol in HIMs and MIMs (Figures 9–10). As expected, glucuronidation occurred extensively in HIMs and MIMs. And experiments of in situ mice single-pass intestinal perfusion model also indicated that glucuronidation of eriodictyol occurred in the intestine.

An intragastrically administered drug is subjected to both intestinal and liver metabolism before it enters the systemic circulation. Nonetheless, a drug administered through intraperitoneal injection undergoes just liver metabolism. We found that intraperitoneal injection but not intragastric administration of eriodictyol (200 mg/kg) attenuates APAP-induced hepatotoxicity. Considering above results, we hypothesized that the protective effect of eriodictyol will probably be related to the eriodictyol exposure. To verify this hypothesis, we performed the PK–PD analyses of eriodictyol administered intragastrically or via intraperitoneal injection before APAP dosing. Intragastrical administration of eriodictyol, which displayed lower parent and higher metabolite concentrations in the plasma, did not elicit protective effects against APAP hepatotoxicity (Figure 12). To confirm the role of UGT metabolism in eriodictyol exposure, we determined the pharmacokinetic behavior of eriodictyol in the presence or absence of GA. GA is an inhibitor with multiple inhibitory effects. It could inhibit the activities of UGT1A1, UGT1A9, UGT1A3 and UGT2B7 by 78.4\%, 72.5\%, 93.6\% and 99.7\%\textsuperscript{37}, respectively. Our results showed that the bioavailability of eriodictyol was increased to 216.84\% in the presence of GA (Figure 13 and Table 3). Therefore, UGT metabolism decreased the extent of eriodictyol exposure \textit{in vivo}. 
Subsequently, we determined the role of GA in the protective effects of eriodictyol and found that intragastric eriodictyol administration combined with GA attenuated APAP-induced hepatotoxicity, although GA and eriodictyol alone did not improve the damage (Figure 14). This result was consistent with previous findings, which indicated that GA, a glycyrrhizin metabolite, did not attenuate APAP hepatotoxicity. Consequently, GA improved the PK–PD behavior of eriodictyol. Ultimately, we used Ugt1+/− mice to determine the active substance responsible for the protective effect of eriodictyol against APAP-induced hepatotoxicity. Consistently, intragastric eriodictyol administration attenuated the APAP-induced hepatotoxicity in Ugt1+/− mice but not in its wild-type littermates.

Our results shown that APAP overdose caused elevation of plasma of TNF-α and IL-1β levels, reduction of hepatic GSH content and SOD activity, and enhancement of MDA level, and ultimately promotion of caspase-3 activity and hepatic cell apoptosis (Figure 16-20). Administration of eriodictyol, these phenomena had been reversed. Firstly, hepatic GSH content was raised by 3.6-fold in the presence of eriodictyol. Secondly, the activity of SOD was enhanced by 1.6-fold. Thirdly, MDA level was decreased 2-fold. It is worth mentioning that eriodictyol could also significantly inhibit the activity of cyp2e1 and cyp3a11 which mediated the formation toxic metabolite of APAP (Figure 18). These findings revealed that eriodictyol, besides retaining hepatic GSH level by improvement of hepatic GSH-Px, GR and GST activities, could provide other anti-oxidative actions like inhibiting cyp2e1 and cyp3a11 and maintaining SOD and MDA to protect liver against APAP. NAC, a cysteine prodrug, replenishes and maintains hepatic GSH by providing cysteine, which detoxifies reactive metabolite of APAP44,45. Additionally, NAC can enhance sulfonation pathway of APAP metabolism to reduce the formation of toxic metabolite46,47. Hence, the protective mechanisms of eriodictyol and NAC against APAP hepatotoxicity are different. However, adverse reactions to NAC treatment during APAP overdose are relatively common, which includes vomiting, diarrhea, skin reactions, and headache48. NAC can be delivered as either intravenous or oral preparations, a loading does of 150 mg/kg and 140 mg/kg, respectively8. Eriodictyol is naturally present in many edible plants including vegetables and fruits. According to Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial
Clinical Trials for Therapeutics in Adult Healthy Volunteers (U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, July 2005), intravenous injection of eriodictyol at 50-200 mg/kg in mice is approximately equal to 4.1-16.3 mg/kg for an adult, which is much less than NAC. Our data indicated that intravenous injection of eriodictyol at 200 mg/kg didn’t increase the ALT, AST levels or cause hepatocellular necrosis or any other toxic sign in control mice. Thus, the application of eriodictyol for alleviation of APAP overdose might be safe and feasible.

In conclusion, parenterally administered eriodictyol may be used to treat APAP-induced hepatotoxicity via retaining hepatic GSH, inhibiting cyp2e1 and cyp3a11 activities and maintaining SOD and MDA levels, and its efficacy can be enhanced by UGT1As down-regulation.
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Abbreviations

GA, glycyrrhetinic acid; IS, internal standard; UGT, uridine-5'-diphospho-glucuronosyltransferase; UDPGA, uridine diphosphoglucuronic acid; HLMs, human liver microsomes; MLMs, mice liver microsomes; HIMs, human intestinal microsomes; MIMs, mice intestine microsomes; M1, eriodictyol’s metabolite 1; M2, eriodictyol’s metabolite 2; Con, concentration; MS, mass spectrometry; ESI, electrospray ionization; Q-TOF, quadrupole time-of-flight; UPLC, ultraperformance liquid chromatography; HRMS, high-resolution mass spectrometry; $V_{\text{max}}$, maximal velocity; $K_m$, substrate concentration at half-maximal rate; $K_{\text{sis}}$, substrate inhibition constant; $CL_{\text{int}}$, intrinsic clearance; $T_{\text{max}}$, time at maximum observed concentration.; $C_{\text{max}}$, maximum observed concentration; $T_{1/2}$, time for concentration to diminish by one-half; AUC, cumulative area under curve; $MRT$, mean residence time; $F_{\text{rel}}$, relative bioavailability; NAPQI, N-acetyl-p-benzoquinone imine; cyp, cytochromes P450; NAC, N-acetyl-cysteine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; GSH, glutathione; GSH-Px, glutathione oxidized; GR, glutathione reductase; GST, glutathione S-transferase; SOD, superoxide dismutase; MDA, malondialdehyde; TUNEL, transferase-mediated deoxyuridine triphosphate-biotin nick end labeling.
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