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## The metabolism of berberine and its contribution to the pharmacological effects

Kun Wang<sup>a,b</sup>, Xinchu Feng<sup>a</sup>, Liwei Chai<sup>a,b</sup>, Shijie Cao<sup>a,b</sup> and Feng Qiu<sup>a,b</sup>

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### ABSTRACT

Berberine, a bioactive alkaloid isolated from several herbal substances, possesses multiple pharmacological effects, including antimicrobial, antidiabetic, anticancer activities. Meanwhile, berberine undergoes extensive metabolism after oral administration which results in its extremely low plasma exposure. Therefore, it is believed that the metabolites of berberine also contribute a lot to its pharmacological effects. Along these lines, this review covers the metabolism studies of berberine in terms of its metabolic pathways and metabolic organs based on the identified metabolites, and it also covers the pharmacological activities of its active metabolites. In brief, the predominant metabolic pathways of berberine are demethylation, demethylenation, reduction, hydroxylation and subsequent conjugation *in vivo*. Active metabolites such as columbamine, berberrubine and demethyleneberberine also exhibit similar pharmacological effects by comparison with berberine, such as antioxidant, anti-inflammatory, antitumor, antimicrobial, hepatoprotective, neuroprotective, hypolipidemic and hypoglycemic effects. Overall, berberine together with its metabolites formed the material basis of berberine *in vivo*.

**Abbreviations:** AMPK: AMP-activated protein kinase; AUC<sub>0-t</sub> h: the area under the concentration-time curves from zero to t h; COX-2: cyclooxygenase-2; CYPs: cytochromes P450s; DhBBR: dihydroberberine; HDL: high-density lipoprotein; HepG2: HepG2 cell line; IC<sub>50</sub>: half maximal inhibitory concentration; IL-1 $\beta$ : interleukin 1 $\beta$ ; iNOS: inducible nitric oxide synthase; i.p.: intraperitoneal injection; IT-TOF/MS: ion trap time-of-flight mass spectrometer; LC-MS/MS: liquid chromatography-mass spectrometer; LD<sub>50</sub>: median lethal dose; LDL-C: low-density lipoprotein cholesterol; LPS: lipopolysaccharide; NADPH: reduced nicotinamide adenine dinucleotide phosphate; NAFLD: nonalcoholic fatty liver diseases; NF- $\kappa$ B: nuclear factor- $\kappa$ B; NMR: nuclear magnetic resonance; NOE: nuclear overhauser effect; PPAR  $\alpha$ : peroxisome proliferator-activated receptors  $\alpha$ ; Q-TOF/MS: quadrupole time-of-flight mass spectrometry; ROS: reactive oxygen species; SOD: superoxide dismutase; SULTs: sulfotransferases; T2DM: type-2 diabetes mellitus; TG: triglyceride; TNF- $\alpha$ : tumor necrosis factor  $\alpha$ ; UGTs: UDP-glucuronosyltransferases

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### Introduction

Berberine is the principal component for many popular medicinal plants (Table 1), such as *Coptidis chinensis* Franch. (family *Ranunculaceae*), *Phellodendron chinense* Schneid. (family *Rutaceae*), and *Mahonia bealei* (Fort.) Carr. (family *Berberidaceae*). These traditional medicines were recorded in the Pharmacopoeia of China (2015) for their excellent efficacy, including clearing away heat, resolving dampness, purging fire and detoxification. Additionally, berberine is also a main bioactive substance used as a chemical marker for quality control of many prescriptions such as Huanglian-Jie-Du-Tang (decoction of *Coptidis rhizoma*, *Scutellariae radix*, *Phellodendri cortex* and *Gardeniae fructus*), Gegen-Huangqin-Huanglian-Tang (decoction of *Puerariae*

*lobatae radix*, *Scutellariae radix*, *Coptidis rhizoma* and *Glycyrrhizae radix*) and Zuo-Jin-Wan (decoction of *Coptidis rhizoma* and *Euodiae fructus*), which have been used in clinical treatment for centuries (Yang et al., 2010). Nowadays, berberine is getting more attention from investigators due to its excellent biological activities, and a growing number of studies focused on berberine have been conducted for its further development.

In clinical practice, berberine is used for the treatment of some clinical disorders, such as type-2 diabetes mellitus (T2DM) (Chen et al., 2013a; Cicero & Tartagni, 2012; Lan et al., 2015; Ni et al., 2015; Pang et al., 2015), neurodegenerative diseases (Ahmed et al., 2015; Ye et al., 2009), cancers (Diogo et al., 2011;

**Table 1.** The known natural sources of berberine and their medical uses.

| Natural sources   | Family        | Medical uses  |
|---|---------------|---|
| <i>Coptis chinensis</i> Franch., <i>Coptis deltoidea</i> C. Y. Cheng et Hsiao, <i>Coptis teeta</i> Wall. (Their crude drugs are known as <i>Coptidis rhizoma</i> )                                  | Ranunculaceae | Treatment of intestinal infections including acute gastroenteritis, cholera and bacillary dysentery (Tang et al., 2009)   |
| <i>Hydrastis canadensis</i> L.  | Ranunculaceae | Treatment of gastrointestinal disturbances, urinary disorders, skin, mouth and eye infections (Hwang et al., 2003)  |
| <i>Coptis japonica</i> Makino   | Ranunculaceae | Treatment of the inflammation-related diseases (Cho et al., 2001)   |
| <i>Berberis vulgaris</i> L.   | Berberidaceae | Treatment of liver dysfunction, kidney pain, diarrhea, indigestion and urinary tract diseases (Laamech et al., 2017)  |
| <i>Berberis aetnensis</i> C. Presl  | Berberidaceae | Unknown (Campisi et al., 2011)  |
| <i>Berberis soulieana</i> Schneid., <i>Berberis poiretii</i> Schneid., <i>Berberis wilsonae</i> Hemsl., and other <i>Berberis</i> species (Their crude drugs are known as <i>Berberidis radix</i> ) | Berberidaceae | Treatment of dysentery, gastroenteritis, toothache, aptha, sore throat, acute hepatitis, chronic cholecystitis, jaundice and dyspepsia (Dan et al., 2011)               |
| <i>Berberis koreana</i> Palib.  | Berberidaceae | Treatment of fever, conjunctivitis, sour throat, diarrhea, parasite infections and trachoma infections (Lee et al., 2010)   |
| <i>Berberis thunbergii</i> DC.  | Berberidaceae | Treatment of enteric infections, particularly bacterial dysentery (Hussain et al., 2017)  |
| <i>Berberis aristata</i> DC.  | Berberidaceae | Treatment of malaria, bleeding, fever, skin and eye infections, jaundice, diarrhea and hepatitis (Balasubramani et al., 2011; Potdar et al., 2012)                      |
| <i>Berberis lyceum</i> Royle  | Berberidaceae | Treatment of diabetes, liver and digestive complaints, coughs and colds, sore eyes, broken bones, ulcers and wounds (Balasubramani et al., 2011; Gulfranz et al., 2008) |
| <i>Berberis asiatica</i> Roxb.  | Berberidaceae | A substitute to <i>Berberis aristata</i> DC. (Balasubramani et al., 2011)   |
| <i>Mahonia aquifolium</i> (Pursh) Nutt.   | Berberidaceae | Treatment of skin diseases and digestive disorders (Brezova et al., 2004; He & Mu, 2015)  |
| <i>Mahonia bealei</i> (Fort.) Carr., <i>Mahonia fortunei</i> (Lindl.) Fedde (Their crude drugs are known as <i>Mahoniae caulis</i> )  | Berberidaceae | Treatment of acute dysentery, icteric hepatitis conjunctivitis, ulcers, boils, carbuncles, toothache (He & Mu, 2015)  |
| <i>Phellodendron chinense</i> Schneid., <i>Phellodendron amurense</i> Rupr. (Their crude drugs are known as <i>Phellodendri cortex</i> )  | Rutaceae      | Treatment of dysentery, jaundice and urinary infection (Chen et al., 2010b)   |
| <i>Chelidonium majus</i> L.   | Papaveraceae  | Treatment of liver diseases, gastric ulcer, oral infections, pain, skin eruptions and tuberculosis (Gilca et al., 2010)   |

Kaboli et al., 2014; Ortiz et al., 2014; Sun et al., 2009) and other diseases (Ji & Shen, 2011; Lau et al., 2006; Wu et al., 2010). Obviously, berberine is a promising active composition from medicinal plants. However, pharmacokinetic studies have indicated that berberine undergoes extensive metabolism after oral administration and its plasma concentration was extremely low. Thus, more and more researchers have focused their attention on the metabolites of berberine, and various studies have proven that the metabolites of berberine play an important role in the treatment of various diseases. In this review, the metabolism of berberine was summarized in detail, including the potential metabolic pathways and the main metabolic organs based on the identified berberine-related metabolites. Additionally, the pharmacological activities of berberine's metabolites were also reviewed.

### Pharmacokinetics of berberine

In recent years, many studies focused on the pharmacokinetic properties (absorption, tissue distribution, metabolism and elimination) of berberine have been conducted (Cui et al., 2015; Liu et al., 2010;

Ma et al., 2013; Tan et al., 2013). Berberine could be absorbed in both rats and human gastrointestinal tract after oral administration. However, its absolute bioavailability is extremely low (Cheng et al., 2016; Liu et al., 2016; Liu & Chen, 2012; Tan et al., 2013; Wu et al., 2015). After a single oral dose of 400 mg berberine in a pilot pharmacokinetic study (Hua et al., 2007), the mean maximum plasma concentration ( $C_{max}$ ) in 20 volunteers was about 0.4 ng/mL, and the area under the concentration-time curves from zero to infinity ( $AUC_{0-\infty}$ ) was only about 9.18 h<sup>-1</sup> ng/mL. Tissue distribution studies indicated that berberine could be widely distributed in rat organs including the liver, kidneys, muscle, lungs, brain, heart, pancreas (Tan et al., 2013; Wang et al., 2015a; Zuo et al., 2006) and the concentration of berberine in most of the studied tissues was higher (or much higher) than that in plasma after 4-h administration (Tan et al., 2013). Additionally, the excretion of berberine in rats after oral administration (200 mg/kg) was also evaluated. After 48 h of administration, 22.8% of administered dose was recovered from bile ( $9.2 \times 10^{-6}\%$ ), urine (0.0939%) and feces (22.7%) in the form of berberine (19.07%) and major metabolites (3.76%) (Ma et al., 2013). In human study, only 0.013% of prototype was eliminated directly

in the urine after oral administration of berberine (0.2 g/day) (Yu et al., 2000). Accordingly, several conclusions could be drawn from the results described earlier: (1) berberine has poor absorption in the gut, and most of the oral dose remained inside the gastro-intestinal lumen, which was excreted in the feces finally. (2) After absorption from the gastrointestinal tract, berberine could be widely distributed in the organs, while its concentration in plasma is low. (3) The part of berberine which was absorbed into body could be converted into multiple metabolites. In fact, berberine and its metabolites exist simultaneously *in vivo*.

## Metabolism of berberine

According to the pharmacokinetic properties described earlier, berberine goes through extensive metabolism after oral administration. In this section, the metabolism of berberine was reviewed in terms of metabolic pathways and metabolic organs based on the identified metabolites.

### Metabolites of berberine identified in plasma, urine, feces, bile and tissues

Metabolite identification plays an important and critical role in drug metabolism studies. A great deal of *in vivo* studies have elucidated the metabolites of berberine, and those publications contributed immensely to understanding the metabolism of berberine *in vivo*, although many metabolites have been identified by LC-IT-TOF/MS (Ma et al., 2013; Tan et al., 2013) or LC-Q-TOF/MS (Guo et al., 2011) system that could only ascertain the chemical formula and the possible structure. So far, more than 20 berberine-related metabolites (Figure 1) have been identified in humans and rats.

In rat studies, plasma profiles of berberine and its metabolites were investigated after intravenous administration (4 mg/kg, berberine). The major circulating metabolites of berberine were berberrubine, demethyleneberberine and their corresponding glucuronides (Liu et al., 2009). Recently, a total of 16 berberine-related metabolites including 10 phase-I metabolites and six phase-II metabolites were identified and clarified by LC-IT-TOF/MS system comparing the retention times and MS fragments of corresponding standard substances after oral administration (200 mg/kg berberine) to rats (Ma et al., 2013). Additionally, gut microbiota acting as a "metabolic organ" might convert berberine into dihydroberberine (dhBBR) that was only found in the feces of rats (Feng et al., 2015). In human studies, early results (0.9 g/day berberine, oral administration) revealed that demethyleneberberine-2-O-sulfate was a

major metabolite of berberine in urine (Pan et al., 2002). In our research (Qiu et al., 2008), human urine samples were collected after oral administration (300 mg/day, berberine chloride). A total of seven metabolites were isolated and elucidated by enzymatic deconjugation and their chemical structures were fully determined by MS, NMR and nuclear overhauser effect (NOE) spectroscopy. For comparison, five berberine-related metabolites were identified in rat urine samples that were collected after oral administration (100 mg/kg, berberine chloride). The results suggested that berberine undergoes similar biotransformation in rats and humans (Qiu et al., 2008).

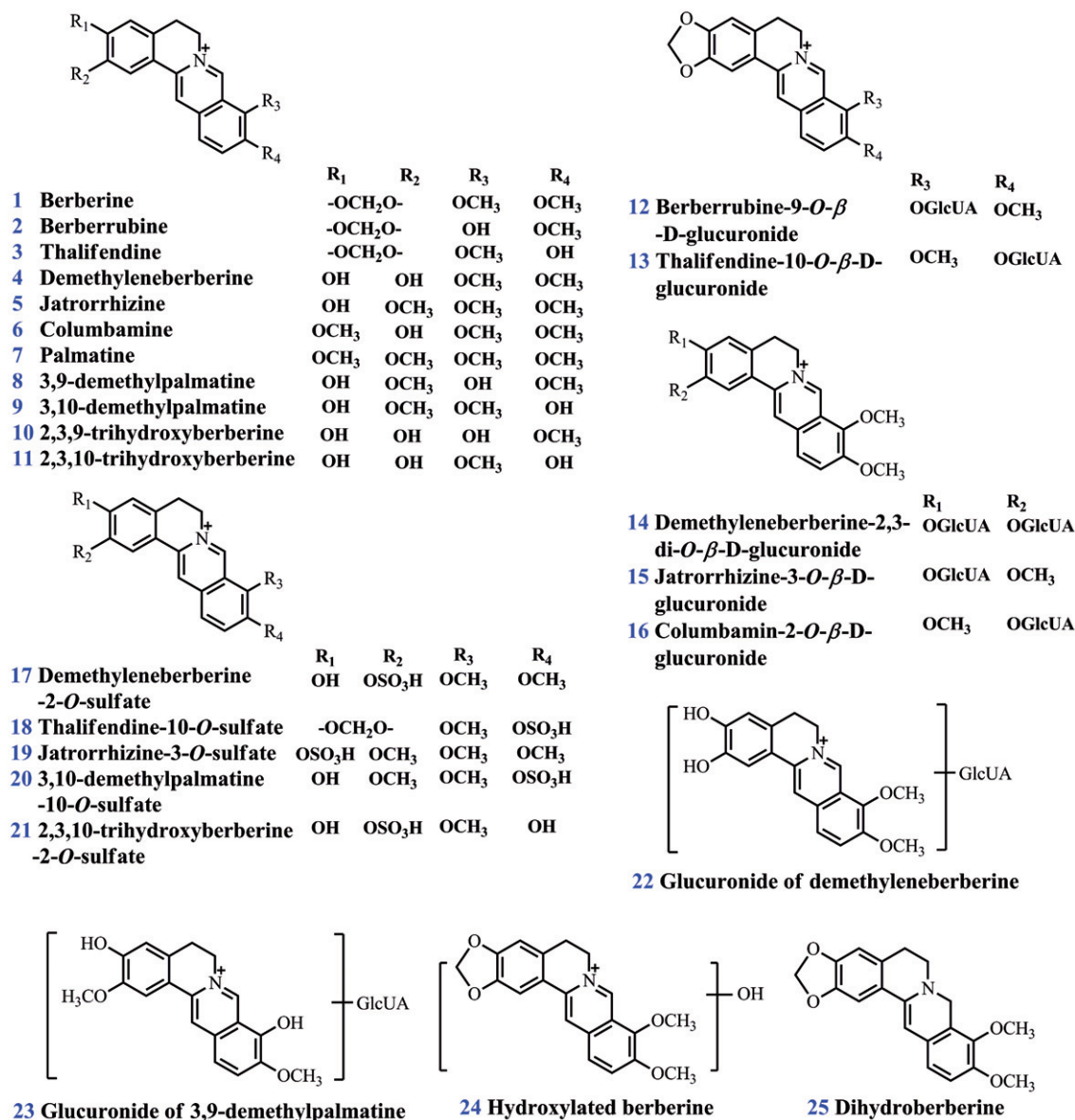
Jatrorrhizine, a major berberine-related metabolite *in vivo*, could be further metabolized to its phase-I metabolites (such as demethylated, dehydrogenated and hydroxylated metabolites) and phase-II metabolites (such as glucuronide conjugates and methylated conjugates) in rats (Han et al., 2006; Zhang et al., 2008). Semiquantitative analysis results (Shi et al., 2012) revealed that glucuronide conjugate and demethylated products of jatrorrhizine were its major metabolites. Moreover, some studies have also found that berberine could be transformed into palmatine *in vivo* (Ma et al., 2013). Likewise, the metabolic profile of palmatine after oral administration to rats was also elucidated (Yang et al., 2009).

### Metabolic pathways of berberine based on the identified metabolites

#### Phase-I metabolism of berberine

Based on the structure of berberine (Figure 2) and the identified structures of metabolites (Figure 1), berberine underwent multiple phase-I metabolic pathways *in vivo* which can be summarized as follows: (1) the methoxyl groups positioned at the D ring (C-9 or C-10) could be demethylated. (2) The dioxymethylene five-membered ring of the structure could be cleaved *via* demethylenation or reduction. (3) The hydroxylation appears to occur at the saturated carbon atom (C-5, C-6) in B ring of the berberine or at the aromatic carbon atoms. (4) Reduction could also take place at C=N double bond in the C ring and finally transformed into C-N single bond.

Demethylation is a major metabolic route for berberine (Chen et al., 2013 b; Guo et al., 2011; Li et al., 2011; Liu et al., 2009; Tan et al., 2013; Tsai & Tsai, 2004; ), which usually occurs at the methoxyl groups at C-9 or C-10 positions of berberine to yield berberrubine and thalifendine (Figure 2), as the main metabolites in rats and humans (Spinuzzi et al., 2014; Tan et al., 2013;



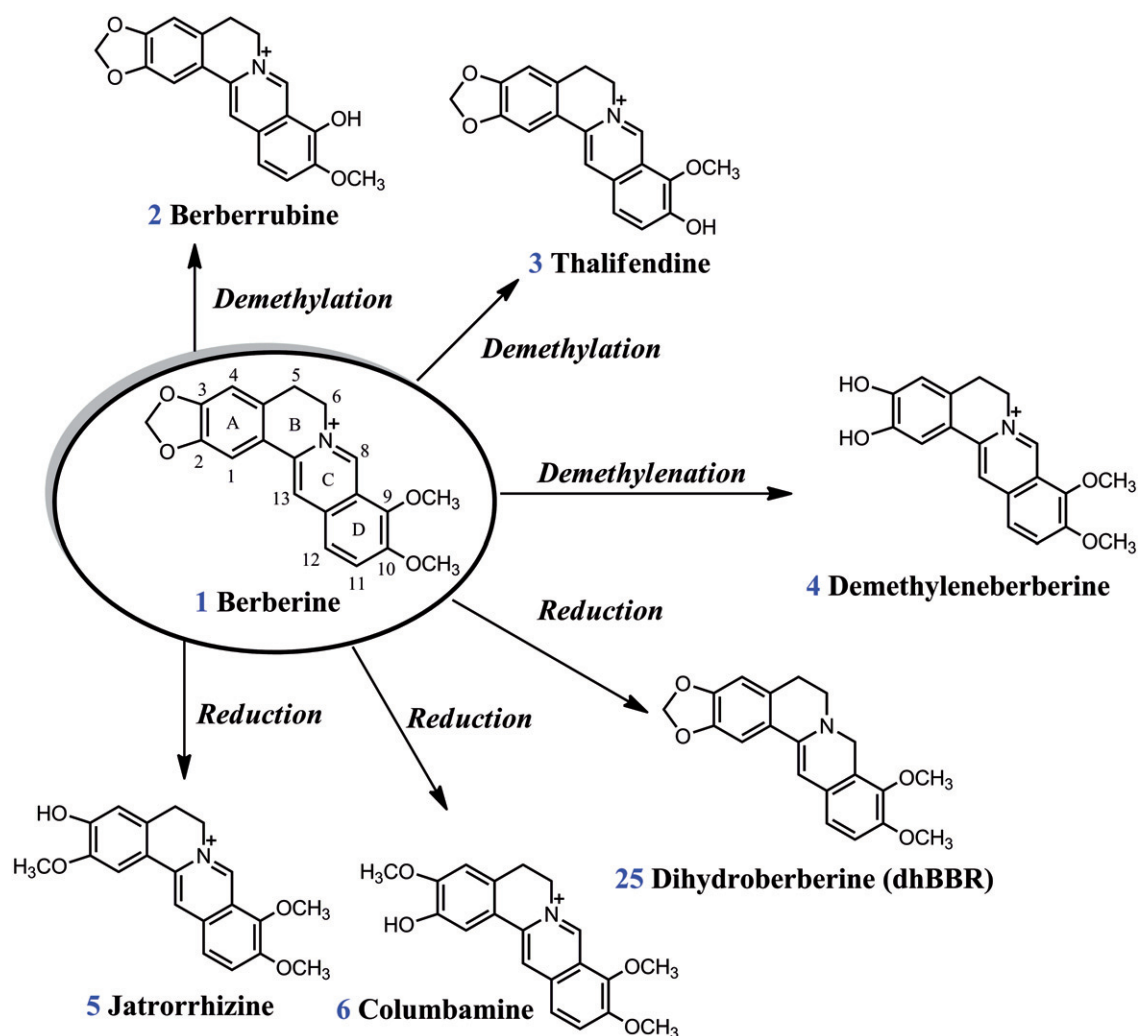
**Figure 1.** The structures of berberine and its metabolites identified *in vivo*.

Zuo et al., 2006). The physicochemical results indicated that berberrubine was more lipophilic and could be absorbed more efficiently in intestine than berberine (Spinuzzi et al., 2014). Meanwhile, some metabolites of berberine that reserve the methoxyl groups such as palmatine (Yang et al., 2009; Zhu et al., 2007) and jatrorrhizine (Zhang et al., 2008) could be further metabolized through mono-, di- or tri-demethylation in rats.

Demethyleneberberine (Figure 2) was another main metabolite of berberine *in vivo*, which was formed by cleavage of the dioxymethylene five-membered ring *via* demethylenation (Chen et al., 2013b; Guo et al., 2011; Liu et al., 2009). Meanwhile, demethyleneberberine was also identified as the demethylated product of jatrorrhizine in rat urine, bile and feces after i.v. administration (3.0 mg/kg) (Shi et al., 2012). The di-demethylation

product with the molecular ions at  $m/z$  324 was also found in rat plasma, urine and feces after administration of a single dose of palmatine (20 mg/kg) by oral gavage, but the authors did not provide further structural evidence to ascertain whether the di-demethylation product of palmatine is demethyleneberberine or not (Yang et al., 2009; Zhu et al., 2007).

Hydroxylation could occur in the structure of berberine, and mono-hydroxylated berberine was identified in rat feces after oral administration (200 mg/kg) (Ma et al., 2013). Hydroxylation, di-hydroxylation or tri-hydroxylation have also been reported as a main metabolic pathway for palmatine (Yang et al., 2009; Zhu et al., 2007) and jatrorrhizine (Han et al., 2006; Zhang et al., 2008). In general, the hydroxylation of the saturated aliphatic ring is easier than that of the aromatic



**Figure 2.** The main phase-I metabolites of berberine *in vivo*.

ring, especially for the saturated carbon atom near to an *ortho*  $sp^2$ -hybridized carbon atom. Since there are two saturated carbon atoms (C-5 and C-6) (Figure 2) in the B ring of berberine, the first hydroxylation position should be the *meta*-saturated carbon of the nitrogen atom at the B ring (C-5), and the subsequent hydroxylation positions might be the *ortho* saturated carbon of the nitrogen atom at the B ring (C-6) and the aromatic carbon atom in the D ring (Yang et al., 2009; Zhang et al., 2008).

Reduction is also an important metabolic pathway of berberine *in vivo*. Jatrorrhizine and columbamine, an isomeric pair (Figure 2), were identified as two hydrogenation metabolites of berberine in humans and rats. They were the dioxymethylene five-membered ring opened products (Guo et al., 2011; Qiu et al., 2008). In addition, dhBBR (Figure 2) is another hydrogenation metabolite, which was found in rat feces and exhibits an intestinal absorption rate higher than that of berberine in animals (Feng et al., 2015). Further study showed

that berberine was converted to dhBBR in a reduction reaction catalyzed by nitroreductase in 14 species of intestinal bacteria, with  $H\cdot$  from FMN-FMN $H_2$  system moved to the C atom of C=N double bond in berberine and finally transformed into C-N single bond. However, dhBBR seemed unstable in solution and could revert to berberine almost completely in intestinal tissues *via* non-enzymatic oxidization within ten minutes (Feng et al., 2015).

Phase-I metabolism was usually mediated by cytochrome P450 enzymes (CYPs) in liver and intestine (Guo et al., 2011). Human recombinant CYPs were used to identify the specific CYPs involved in phase-I metabolism of berberine and the results indicated that CYP2D6 was the primary enzyme involved in the formation of the demethylated and demethylenated products. CYP1A2 produced the same metabolites at a slower rate while CYP3A4, 2E1 and 2C19 only participated in the formation of the demethylenated metabolite (Guo et al., 2011). Accordingly, quinidine (the specific

inhibitor of CYP2D6) and furafylline (the specific inhibitor of CYP1A2) could significantly inhibit berberine metabolism (Chen et al., 2013b). Similarly, cell-free reactions also showed that CYP2D6, CYP1A2 and CYP3A4 were the dominant CYP450 isoenzymes transforming berberine into its metabolites. CYP2D6 and CYP1A2 play a major role in transforming berberine into thalifendine, as well as CYP2D6, CYP1A2 and CYP3A4 for demethyleneberberine production (Li et al., 2011).

Furthermore, based on recent publications, jatrorrhizine and palmatine, two metabolites for berberine *in vivo*, might be further metabolized by CYPs. *In vitro* metabolism studies, CYP3A1/2 and CYP2D2 in rat liver microsomes (Shi et al., 2012) and CYP1A2 in human liver microsomes (Zhou et al., 2013) could catalyze the *O*-demethylation of jatrorrhizine, while human recombinant CYP2D6 and CYP1A2 play a major role in *O*-demethylation of palmatine (Vrba et al., 2015).

### Phase-II metabolism of berberine

Phase-II reactions, known as conjugation reactions with glucuronic acid, sulfuric acid, or methyl groups have been reported in previous studies (Chen et al., 2010a; Yang et al., 2009). After oral administration, berberine was rapidly metabolized to phase-I products, which were further conjugated with glucuronic acid or sulfuric acid to form phase-II metabolites rapidly and finally excreted in the urine and bile (Ma et al., 2013).

Glucuronidation of berberine is mainly the formation of glucuronide conjugates with its phase-I metabolites such as berberrubine, demethyleneberberine, jatrorrhizine and columbamine (Guo et al., 2011; Liu et al., 2009; Tsai & Tsai, 2004). In general, berberine phase-I metabolites are mainly converted into glucuronide conjugates. For instance, jatrorrhizine-3-*O*-glucuronide is the predominant metabolite for jatrorrhizine in rat plasma after intravenous administration (0.1, 0.3, 3.0 mg/kg), because the glucuronidation product was the most abundant compared with its other metabolites (Shi et al., 2012). Glucuronidation was mediated by UDP-glucuronosyltransferases (UGTs) including UGT1, 2, 3 and 8 families. Among them, UGT1 and UGT2 are the most predominant subclasses involved in drug metabolism (Chen et al., 2014). Berberrubine and demethyleneberberine, for instance, could be glucuronidated by UGT1A1 and UGT2B1, while the glucuronidation of demethyleneberberine was favored by UGT1A1 (Liu et al., 2009). Meanwhile, UGT1A1 and UGT1A3 in rat liver microsomes (Shi et al., 2012) and UGT1A1, UGT1A3, UGT1A7, UGT1A8, UGT1A9 and UGT1A10 in human liver microsomes (Zhou et al., 2013) were responsible for the glucuronidation of jatrorrhizine.

Generally, sulfation could terminate the biological activity of drugs, and the solubility of these metabolites is increased markedly, which could benefit their excretion. Like glucuronidation, the sulfation of berberine (Guo et al., 2011; Pan et al., 2002) was also subsequent conjugation with its phase-I metabolites. Sulfation is catalyzed by sulfotransferases (SULTs), which are located in the hepatic and intestinal cytosol (Wang & Qiu, 2013). SULTs, divided into four families including SULT1, 2, 4 and 6, mainly transfers a sulfo group ( $\text{SO}_3\text{H}^-$ ) from 3'-phosphoadenosine-5'-phosphosulfate to substrates (Chen et al., 2014). Several sulfated metabolites of berberine were found in humans and rats, such as jatrorrhizine-3-*O*-sulfate, demethyleneberberine-2-*O*-sulfate and thalifendine-10-*O*-sulfate (Pan et al., 2002). However, further investigations are needed to clarify which kinds of SULT isoenzymes were involved.

Methylation is a common metabolic pathway for drugs *in vivo*. Similarly, it is a main phase-II metabolic route of jatrorrhizine (Shi et al., 2012). There are two isoforms of catechol-*O*-methyltransferase (COMT) to catalyze methylation in humans (Chen et al., 2014). Usually, berberine could be metabolized to jatrorrhizine, which could be subsequently transformed into palmatine through methylation *in vivo*. Meanwhile, palmatine was also confirmed as a metabolite after berberine oral administration (200 mg/kg) to rats (Ma et al., 2013).

To sum up, demethylation, demethylenation, reduction and subsequent conjugation with glucuronic acid and sulfuric acid are the major metabolic pathways for berberine in rats or humans. The possible metabolic pathways for berberine based on the identified metabolites in humans and rats are described in Figure 3(a,b), respectively.

### Metabolic organs for berberine based on the identified metabolites

#### Hepatic metabolism of berberine

After intravenous administration to rats, the metabolism of berberine occurred primarily in the liver (Liu et al., 2009; Tsai & Tsai, 2004) and demethylenation, demethylation, and their phase-II glucuronidation happened. Meanwhile, after oral administration to rats, berberine together with four major metabolites (berberrubine, thalifendine, demethyleneberberine and jatrorrhizine) and their respective glucuronide conjugates were detected in liver tissues at 0.5 h. Thus, researchers concluded that the liver seemed to be the main metabolic site for berberine (Zuo et al., 2006).

Incubations with liver microsomes, hepatic S9 fractions, isolated hepatocytes, and hepatic cell lines are



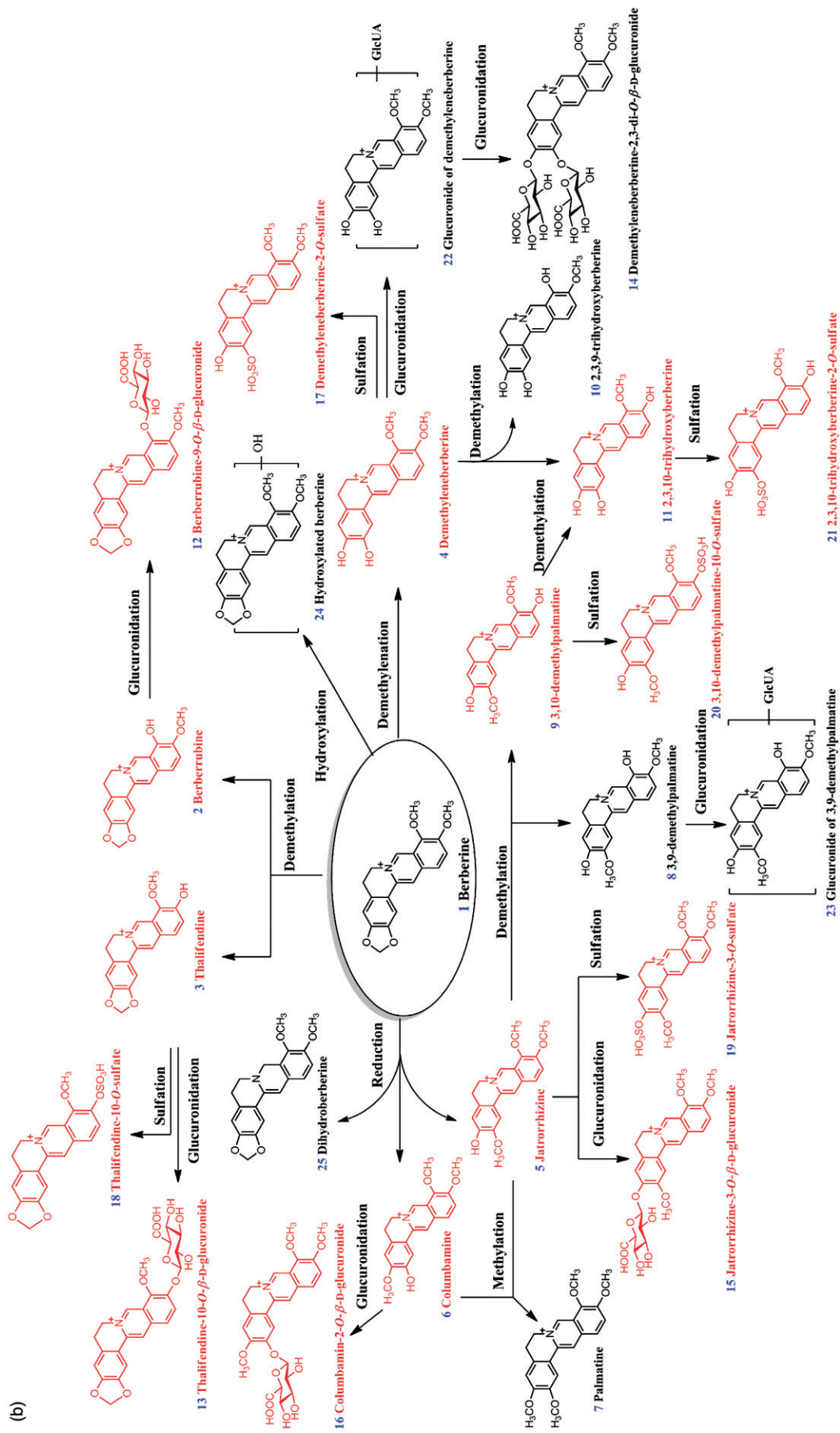


Figure 3. Continued.

widely used to study the hepatic metabolism of drugs (Ma et al., 2014). Fresh liver samples were homogenized on ice, thoroughly mixed with berberine, and then incubated at 37 °C for 4 h. Four phase-I metabolites (berberrubine, thalifendine, demethyleneberberine, jatrorrhizine) were detected (Zuo et al., 2006). *In vitro* incubations with human or rat liver microsomes by addition of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) generating system, berberrubine, demethyleneberberine and jatrorrhizine were generated (Liu et al., 2009; Thomson & Winder, 2009). In addition, the reaction mixture, which consisted of berberrubine or demethyleneberberine, was incubated by adding uridine diphosphate glucuronic acid (UDPGA). The subsequent glucuronidation could happen, while the formation of demethyleneberberine glucuronide was much faster than that of berberrubine glucuronide (Liu et al., 2009). Incubations with liver S9 fraction, berberrubine, demethyleneberberine, jatrorrhizine and their corresponding glucuronides could also be formed, but the reactions occurred at a lower rate compared with the liver microsomes (Liu et al., 2009).

### Intestinal metabolism of berberine

Berberine was administered to rats via four different dosing routes (intragastric, intraduodenal, intraportal and intravenous) to investigate its intestinal first-pass metabolism. The area under the concentration–time curves from zero to  $t$  h ( $AUC_{0-t\ h}$ ) and  $AUC_{0-\infty}$  were calculated by the linear trapezoidal method (Liu et al., 2010). The  $AUC_{0-t\ h}$  values of berberine after intragastric administration were not significantly different from that after intraduodenal dosing ( $57.9 \pm 20.6$  versus  $59.3 \pm 17.0$  ng·h/mL based on 100 mg/kg), indicating that the gastric first-pass elimination was almost negligible in rats. However, the  $AUC_{0-t\ h}$  values of berberine after intraduodenal (i.d.) dosing were significantly less than those after intraportal (i.p.v.) administration (mean  $AUC_{i.d.}$  was nearly 0.5% of the  $AUC_{i.p.v.}$  when normalized by the dose given), suggesting that the intestinal first pass metabolism for berberine was tremendous in rats (Liu et al., 2010). In addition, the intestinal metabolism of berberine has been studied using intestinal post-mitochondrial (S9), cytosol or microsomes fractions, according to the methods described by Ma et al. (2014). Five metabolites (identified as berberrubine, demethyleneberberine, jatrorrhizine, the glucuronide of berberrubine and the glucuronide of demethyleneberberine) were generated in enterocyte fractions S9 and intestinal perfusates *in vitro* studies, and formation velocity results revealed that berberrubine, the glucuronide of

demethyleneberberine and jatrorrhizine are the major metabolites from the small intestine (Liu et al., 2010).

### Microbial metabolism of berberine

The intestinal microbiota is closely involved in the first-pass metabolism of orally administered substances, and the complex microbial ecosystem in the gastrointestinal tract (GI) was considered as a “hidden organ”, with metabolic capacities similar to the liver and the intestine (Feng et al., 2015). Previous studies suggested that berberine is relatively stable in the gastrointestinal tract (Qiu et al., 2008), and the intestinal flora had no significant metabolic activity against berberine and its major metabolites but might play a significant role in the enterohepatic circulation of the metabolites (Zuo et al., 2006). However, recent research has found dhBBR in the feces of rats treated with berberine (200 mg/kg, orally), and the authors speculated that dhBBR was a berberine-related metabolite generated in the intestinal ecosystem (Feng et al., 2015). After *in vitro* incubation with berberine (50 µg/mL) using 14 intestinal bacterial strains, berberine was converted to dhBBR which displayed significantly improved absorption as compared with berberine in the Caco-2 cell model (Feng et al., 2015). Meanwhile, several berberine-related metabolites including berberrubine, demethyleneberberine and jatrorrhizine were also found after anaerobic incubation with berberine using the rat gut microbiota (Li et al., 2014).

In summary, the metabolism in small intestine and liver after absorption is a major reason for low plasma concentrations of berberine *in vivo*. Meanwhile, the unabsorbed dose will enter the intestinal tract and interact with the gut bacteria. As a result of biotransformation, the structure of berberine was changed and new metabolites were generated, which could exhibit similar pharmacological activities *in vivo*.

### Biological activities of the metabolites of berberine

Even though berberine possesses a really low oral bioavailability, it has exhibited marked biological activities *in vivo* and the concentrations of its major metabolites such as berberrubine, thalifendine, demethyleneberberine and jatrorrhizine were at relatively high levels (Caliceti et al., 2016; Zuo et al., 2006). These reports indicated that the metabolites of berberine may be active constituents which are representative for the biological activities of berberine *in vivo*. In fact, many studies have already revealed that the metabolites of berberine showed similar bioactivities (Spinozzi et al., 2014).

For example, both berberine and its metabolites have hypolipidemic effects (Zhou et al., 2014), and columbamine could exhibit marked potential effects on triglyceride (TG)-lowering among them (Cao et al., 2013). Moreover, jatrorrhizine exhibited antimicrobial (Yan et al., 2008), hypolipidemic (He et al., 2016) and hypoglycemic (Fu et al., 2005) effects. In the present review, the bioactivities of the major metabolites of berberine have been summarized (Table 2) in a comprehensive way.

### Antioxidant activities

Oxidative stress is a deleterious process that can be an important mediator of damage to cell structures and consequently induces various disease states, such as cardiovascular disease, cancer, neurological disorders and diabetes. The overproduction of reactive oxygen species (ROS), most frequently either by excessive stimulation of NAD(P)H via cytokines or by the mitochondrial electron transport chain and xanthine oxidase, could result in oxidative stress (Valko et al., 2007). The hydroxyl radical ( $\cdot\text{OH}$ ) is the most reactive product of ROS. The  $\cdot\text{OH}$  scavenging activity of berberrubine, a major metabolite of berberine, was investigated using electron spin resonance spectrometry method. At the concentration of 1 mM, berberrubine and berberine showed excellent  $\cdot\text{OH}$  scavenging activity with 85% and 23%, respectively (Jang et al., 2009). Further results indicated that the  $\cdot\text{OH}$  scavenging activity of berberrubine was closely related to its ferrous ion chelating activity and that the hydroxyl group at C-9 of berberrubine was an essential moiety (Jang et al., 2009).

The antiradical and antioxidant activities of jatrorrhizine, a berberine's metabolite *in vivo*, were also studied using the free stable  $\alpha, \alpha'$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical system and the heterogeneous membrane system of dioleoyl phosphatidylcholine (DOPC) liposomes stressed by peroxidative damage induced using 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH). The tested compounds were used at the dose of 33.3  $\mu\text{M}$  for DPPH system and at the dose of 60  $\mu\text{M}$  for DOPC system, respectively, and the results indicated that jatrorrhizine which has free phenolic group, showed better antioxidant activities than berberine in both systems (Rackova et al., 2004).

### Hepatoprotective effects

Demethyleneberberine is an essential metabolite of berberine. Recent reports have revealed that demethyleneberberine showed hepatoprotective and antifibrotic effects of demethyleneberberine *in vivo*

(Wang et al., 2016). On the thioacetamide (TAA)-induced hepatic fibrosis model in mice, demethyleneberberine (10, 20 mg/kg, i.p.) was able to suppress the activation of hepatic stellate cells and induce cell apoptosis through the nuclear factor kappa B (NF- $\kappa$ B) cascade (Wang et al., 2016).

Excessive alcohol consumption induces oxidative stress and lipid accumulation in the liver, and mitochondria have long been recognized as the major targets of ethanol-induced oxidative stress (Zhang et al., 2014). In the study conducted by Zhang et al., demethyleneberberine showed significant hepatoprotective effect in acute ethanol-treated mice. After the demethyleneberberine treatment (40 mg/kg per day, i.p.), the histopathological damages including microsteatosis, swelling, and apoptosis in liver cells of binge-drinking mice were remarkably attenuated. Meanwhile, demethyleneberberine could reduce the induction of Cytochrome P450 2E1 (CYP2E1), which has been well documented to be a central pathway that contributes to ethanol-mediated oxidative stress *via* reducing the total CYP2E1 protein expression and blocking the distribution of CYP2E1 around the vein. In addition, inducible nitric oxide synthase (iNOS) induced by chronic alcohol consumption in mice which will exacerbate oxidative stress and mitochondrial dysfunction in the liver was also suppressed after demethyleneberberine treatment (Zhang et al., 2014).

Additionally, in a study conducted in methionine and choline deficient high-fat diet feeding (MCD-fed) mice and db/db mice, demethyleneberberine could alleviate nonalcoholic fatty liver diseases (NAFLD) and prevent the pathologic progression from NAFLD to nonalcoholic steatohepatitis (NASH) (Qiang et al., 2016). After demethyleneberberine treatment (20 or 40 mg/kg, i.p.), the hepatic lipid accumulation in MCD-fed mice and db/db mice were decreased (Qiang et al., 2016). Mechanism studies revealed that this was associated with AMPK, which plays an important role in liver lipid metabolism. Demethyleneberberine could activate AMPK by upregulation of its phosphorylation *in vitro* and *in vivo* (Qiang et al., 2016). In addition, demethyleneberberine treatment remarkably attenuated hepatic oxidative stress of MCD-fed and db/db mice, and sharply reduced the inflammation state in NAFLD mice, which was reflected by decreased lipid oxidative product, malonaldehyde and inflammatory factors, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin 1 $\beta$  (IL-1 $\beta$ ) (Qiang et al., 2016).

In addition, berberrubine also possesses hepatoprotective activity by inhibiting the lipid peroxidation, which was induced through  $\text{Fe}^{2+}$  and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in liver homogenate. The half maximal inhibitory concentration ( $\text{IC}_{50}$ ) for the inhibition of

**Table 2.** The activities of berberine metabolites based on available literature.

| Metabolites   | Activities                  | Concentration or dose and experimental model (Reference)                                   | Mechanism  |  |
|---------------|-----------------------------|--|--|--|
|               |                             |  | Inhibition or downregulation   | Activation or upregulation   |
| Berberubine   | Antioxidant activities      | 1 mM and the Fe (II)/H <sub>2</sub> O <sub>2</sub> system (Jang et al., 2009)              | Lipid peroxidation   | The hydroxyl radical scavenging  |
|               | Hepatoprotective properties | 10–100 µg/mL and liver homogenate (El-Salam et al., 2015)                                  | –  | –  |
|               | Hypolipidemic effects       | 15 µM and HepG2 cells (Cao et al., 2013)   | –  | AMPK signaling pathway; low-density lipoprotein receptor expression; Glucose uptake, glycogenesis, the mRNA levels of glucose-6-phosphatase and hexokinase |
|               | Hypoglycemic effects        | 0.6 µM and HepG2 cells (Chen et al., 2012)   | –  | –  |
|               | Antitumor activities        | 5, 20, 50 µM & human normal liver L-O2 cell line (Yang et al., 2017)                       | Topoisomerase II α protein level; topoisomerase II α mRNA level  | –  |
|               | Anti-inflammatory effects   | 100 µM and the topoisomerase II cleavage reactions (Kim et al., 1998)                      | –  | –  |
|               | Hypoglycemic effects        | 20 µM, 50 µM, 100 µM and human colorectal carcinoma cells AMC/B1 line (Kang & Chung, 2002) | Interleukin-8 expression; MCP-1 expression; NF-κB translocation  | –  |
|               | Hypoglycemic effects        | 1–25 µM and ARPE-19 cells (Cui et al., 2006)   | –  | –  |
|               | Hypoglycemic effects        | 5, 20, 50 µM and human normal liver L-O2 cell line (Yang et al., 2017)                     | –  | Glucose consumption, glycogenesis, the mRNA levels of glucose-6-phosphatase and hexokinase   |
|               | Hepatoprotective effects    | i.p., 40 mg/kg per day and binge-drinking mice (Zhang et al., 2014)                        | Microsteatosis; swelling; apoptosis; CYP2E1 protein expression; INOS; the activation of hepatic stellate cells | The phosphorylation of AMPK  |
| Jatrorrhizine | Hypolipidemic effects       | 15 µM and HepG2 cells (Cao et al., 2013)   | –  | AMPK-signaling pathway; low-density lipoprotein receptor expression  |
|               | Antioxidant activities      | 3.3 µM and DPPH system (Rackova et al., 2004)  | Peroxidation of dioleoyl phosphatidylcholine liposomes;  | –  |
|               | Neuroprotective effects     | 60 µM & DOPC system (Rackova et al., 2004)   | The neuronal cell apoptosis by preventing the activation of caspase-3  | –  |
|               | Hypolipidemic effects       | 0.01–10.0 µM and PC12 cells (Luo et al., 2011)   | –  | –  |
|               | Hypolipidemic effects       | 1–10 µM and cortical neurons (Luo et al., 2016)  | –  | –  |
|               | Hypoglycemic effects        | 5, 10 µM and HT22 cells (Jiang et al., 2016)   | –  | AMPK signaling pathway, low-density lipoprotein receptor expression; The mRNA and protein expression of CYP7A1   |
|               | Hypoglycemic effects        | 15 µM and HepG2 cells (Cao et al., 2013)   | p.o., 70.05 mg/kg and <i>Mesocricetus auratus</i> (Wu et al., 2014)  | The activity of succinate dehydrogenase (SDH) and aerobic glycolysis; glucose uptake   |
|               | Hypoglycemic effects        | 0.6 µM and HepG2 cells (Chen et al., 2012)   | –  | –  |
|               | Hypoglycemic effects        | p.o., 10, 20 and 40 mg/kg and normal rats (Patel & Mishra, 2011)                           | –  | –  |
|               | Antitumor activities        | p.o., 50 and 100 mg/kg and normal and hyperglycemic mice (Fu et al., 2005)                 | –  | The cell cycle-suppressive genes <i>p21</i> and <i>p27</i>   |
| Columbamine   | Antimicrobial activities    | 47.6 µM (IC50) and C8161 cell (Liu et al., 2013)   | The growth of <i>Staphylococcus aureus</i>   | –  |
|               | Anti-inflammatory effects   | 200 and 400 µg/mL and <i>Staphylococcus aureus</i> (Yu et al., 2007)                       | –  | –  |
|               | Hypolipidemic effects       | 100 µg/mL and Raw264.7 cells (Cho, 2011)   | The expression of iNOS and COX-2   | –  |
|               | Hypoglycemic effects        | 15 µM and HepG2 cells (Cao et al., 2013)   | –  | AMPK signaling pathway; low-density lipoprotein receptor expression; Glucose uptake  |
|               | Antitumor activities        | 0.6 µM and HepG2 cells (Chen et al., 2012)   | –  | –  |
|               | Antitumor activities        | 21.31 µM (IC50) and U2OS cells (Bao et al., 2012)  | CDK6 gene expression; STAT3 phosphorylation  | –  |

(continued)

Table 2. Continued

| Metabolites | Activities                | Concentration or dose and experimental model (Reference)                                       | Mechanism   |   |
|-------------|---------------------------|--|---|---|
|             |                           |  | Inhibition or downregulation  | Activation or upregulation  |
| Palmitine   | Hypolipidemic effects     | p.o., 23.35, 46.70 and 70.05 mg/kg and hamsters fed with high-fat diet (Ning et al., 2015)     | Expression of mRNA and protein of ASBT  | Expression of mRNA and protein of LDLR and CYP7A1;<br>Fecal excretion of TC and TBA |
|             | Hypoglycemic effects      | p.o., 10, 20, and 40 mg/kg and normal rats (Patel & Mishra, 2011)                              | -   | Expression of PPAR $\alpha$ and mRNA expression of Glut-4; glucose uptake           |
|             | Antimicrobial activities  | 200 and 400 $\mu$ g/mL and L6 cell line (Sangeetha et al., 2013)                               | The growth of <i>Staphylococcus aureus</i>  | -   |
|             | Antitumor activities      | 10, 20 $\mu$ g/mL & C4-2B, PC-3 and DU145 human prostate cancer cells (Hambright et al., 2015) | Activation of NF- $\kappa$ B and its downstream target gene FLIP;<br>tumor invasion | -   |
|             | Anti-inflammatory effects | 80 $\mu$ g/mL and goat endometrial epithelial cells (Yan et al., 2017)                         | tumor necrosis factor- $\alpha$ ; nitric oxide; IL-1 $\beta$ , IL-6                 | IL-10 and pro-resolution mediator   |

berberrubine was of 9.8  $\mu$ g/mL, and it could also decrease thiobarbituric acid reactive species (TBARS) level in a dose-dependent manner due to the antioxidant activity (El-Salam et al., 2015).

### Neuroprotective effects

The neuroprotective activities of berberine's metabolites were usually investigated in neuronal cells. It had been reported that jatrorrhizine showed significant neuroprotective effects in rat pheochromocytoma PC12 cell line due to its antioxidative potential and the inhibition against apoptosis (Luo et al., 2011, 2016). H<sub>2</sub>O<sub>2</sub>, a by-product of enzymatic action and dopamine oxidation, could cause neuronal cell injury and even death. After preincubation of PC12 cells with jatrorrhizine (0.01–10.0  $\mu$ M) for 24 h prior to H<sub>2</sub>O<sub>2</sub> exposure, jatrorrhizine showed significant protective effects against H<sub>2</sub>O<sub>2</sub>-induced injury on PC12 cells and prevented the decrease of antioxidant enzyme (superoxide dismutase (SOD) and heme oxygenase-1) (Luo et al., 2011). Similarly, a significant reduction in cell viability and activities of SOD and glutathione peroxidase (GSH-Px) could occur after cortical neurons were exposed to 25  $\mu$ M A $\beta$  25–35 for 24 h, but pretreatment of cortical neurons with various concentrations of jatrorrhizine (1–10  $\mu$ M) could attenuate A $\beta$  25–35-induced neurotoxicity markedly (Luo et al., 2016). The cytotoxicity and apoptosis induced by okadaic acid (OA) in mouse hippocampal HT22 cells is another model used to study the neuroprotective effects of different compounds. It had been demonstrated that jatrorrhizine could attenuate the reduction in cell survival, activities of SOD, GSH-Px and mitochondria membrane potential (MMP) induced by 80 nM OA for 12 h (Jiang et al., 2016). Moreover, jatrorrhizine (5, 10  $\mu$ M for 12 h) could suppress the H<sub>2</sub>O<sub>2</sub>, A $\beta$  25–35 and/or OA-induced neuronal cell apoptosis by preventing the activation of caspase-3, which is the final executor of apoptosis. (Luo et al., 2011; Luo et al., 2016; Jiang et al., 2016).

### Hypolipidemic effects

Dyslipidemia is a major risk factor for cardiovascular diseases that are the leading cause of death in the world and it is characterized by increased total cholesterol, triglyceride and low-density lipoprotein cholesterol (LDL) levels, and also by declined high-density lipoprotein (HDL) levels (He et al., 2016). AMPK is considered as a key factor for controlling intracellular lipids and glucose metabolism (Thomson & Winder, 2009), and once activated, AMPK switches off anabolic pathways such as cholesterol, fatty acid and triglyceride biosynthesis, as

well as switches on adenosine triphosphate (ATP)-producing catabolic pathways, such as fatty acid oxidation.

In Cao's study, the TG-lowering activities of berberine's metabolites including jatrorrhizine, columbamine, berberrubine and demethyleneberberine were examined using the HepG2 cells (Cao et al., 2013). After the HepG2 cells were treated with four metabolites and berberine (15  $\mu$ M, for 24 h), AMPK-signaling pathways were activated and the intracellular TG contents significantly decreased. Compared with control, columbamine and berberine exhibited high inhibitory rates on TG contents (65% and 55.7%, respectively). Moreover, both columbamine and berberine significantly inhibited the expression of genes responsible for lipogenesis biosynthesis in HepG2 cells such as acetyl-CoA carboxylase (FAS), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), acetyl-CoA carboxylase (ACC) and glycerol-3-phosphate acyltransferase (GPAT) and increased the expression of genes of medium chain acyl-CoA dehydrogenase (mCAD) associated with fatty acid oxidation. These findings suggested that columbamine displays great hypolipidemic roles via regulating the expression of genes involved with lipid biosynthesis and fatty acid oxidation (Cao et al., 2013). In another study, jatrorrhizine, columbamine, berberrubine and demethyleneberberine exhibited lipid-lowering effects by upregulating the low-density lipoprotein receptor (LDLR) mRNA and protein expression, and exhibited effect on reduction of the cellular lipid accumulation in HepG2 cells at 15  $\mu$ M for a 24-h treatment (Zhou et al., 2014).

Other studies also found that oral administration of jatrorrhizine (70.05 mg/kg, for 3 months) to *Mesocricetus auratus* could increase high-density lipoprotein cholesterol (HDL-C) levels and decrease total cholesterol (TC), TG and low-density lipoprotein cholesterol (LDL-C) levels by 20%, 43%, and 19% respectively, via upregulating the mRNA and protein expression of LDLR and cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) (Wu et al., 2014). Similar results were also reported for palmatine in hamsters fed with high-fat diet (HFD). Compared with the HFD group, palmatine treatment (70.05 mg/kg, orally) decreased TC, TG, and LDL-C levels, as well as increased fecal excretion of TC and total bile acids (TBA) in hyperlipidemic hamsters via up-regulating the expression of mRNA (by 2.3-fold and 2.0-fold) and protein (by 1.7-fold and 1.8-fold) of LDLR and CYP7A1, downregulating apical sodium-dependent bile salt transporter (ASBT) mRNA (by 44%) and protein (by 49%) expression, as well as enhancing fecal excretion of TC (by 41%) and TBA (by 70%) (Ning et al., 2015).

In summary, when berberine was given to patients, its metabolites including palmatine, jatrorrhizine,

columbamine, berberrubine and demethyleneberberine might also be active forms together with itself to exert the hypolipidemic effects *in vivo*.

### Hypoglycemic effects

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose level (hyperglycemia) owing to failure of the pancreatic  $\beta$  cells to produce enough insulin, or loss of an effective target tissue response to insulin (Yang et al., 2014). In type-1 or insulin-dependent diabetes, the pancreas fails to secrete insulin. In contrast, T2DM was formerly known as non-insulin-dependent diabetes (Hamid et al., 2015). Many publications have shown that berberine exhibited marked antidiabetic effects on both human beings and type-2 diabetic rodent models (Cicero & Tartagni, 2012; Lan et al., 2015; Pang et al., 2015). As for its metabolites, the hypoglycemic effects were also confirmed in different experimental models. In HepG2 cells, berberrubine, jatrorrhizine and columbamine (0.6  $\mu$ M) exhibited glucose lowering activity with a dose-dependent manner (Chen et al., 2012). Moreover, oral administration of palmatine and jatrorrhizine (10, 20 and 40 mg/kg) significantly decreased fasting serum glucose and suppressed the increase of blood glucose levels after 2 g/kg glucose loading in normal rats (Patel & Mishra, 2011).

Berberrubine is the primary metabolite of berberine and could be quickly metabolized into berberrubine-9-O- $\beta$ -D-glucuronide in rats after oral administration. In human normal liver L-O2 cells *in vitro*, treatment with berberrubine or berberrubine-9-O- $\beta$ -D-glucuronide (5, 20, 50  $\mu$ mol/L) increased glucose consumption, enhanced glycogenesis and modulated the mRNA levels of glucose-6-phosphatase and hexokinase (Yang et al., 2017). These results suggested that berberrubine and berberrubine-9-O- $\beta$ -D-glucuronide were potential agents for reducing glucose levels.

Alloxan can kill  $\beta$  cells in pancreas islets specifically and then induce hyperglycemia. Daily oral administration of jatrorrhizine (50 and 100 mg/kg) could significantly decrease blood glucose after 1, 3, 5 days of treatment in alloxan-diabetic mice in a dose- and time-dependent manner, and enhance the activity of succinate dehydrogenase (SDH), a rate-limiting enzyme in aerobic glycolysis, indicating that pronounced decrease of blood glucose in normal and hyperglycemic mice by jatrorrhizine may be attributed to the enhancement of aerobic glycolysis (Fu et al., 2005).

Muscle tissue is the major site for insulin-stimulated glucose uptake as it expresses insulin-sensitive glucose transporter (Glut-4). Meanwhile, peroxisome proliferator-activated receptors  $\alpha$  (PPAR $\alpha$ ) are also highly

expressed in muscle tissue, which regulates the expression of genes involved in lipoprotein metabolism thereby improving HDL and affecting glucose metabolism (Sangeetha et al., 2013). Exposure of myocytes (L6 skeletal muscle cell line) to palmatine (625 nM for 6 h), the protein expression of PPAR $\alpha$  and mRNA expression of Glut-4 could increase, which indicates that the ability of palmatine to improve cellular glucose uptake is through the upregulation of Glut-4 expression and PPAR $\alpha$  expression (Sangeetha et al., 2013).

## Antitumor activities

### Potential role for topoisomerase II

Topoisomerase II is the intracellular target for a variety of active agents currently used in the treatment of human cancers (Kang & Chung, 2002). The antitumor activity of berberrubine was first found using experimental tumors in mice, and the structure-activity relationship study suggested that the hydroxyl group at the C-9 position of berberrubine is essential for its antitumor activity (Ikekawa & Ikeda, 1982; Kim et al., 1998). Further studies (Kang & Chung, 2002) indicated that topoisomerase II $\alpha$  was the cellular target of berberrubine, which was seen as a specific poison of DNA topoisomerase II by stabilizing topoisomerase II-mediated cleavable complex *in vitro* (Kim et al., 1998).

### Inhibition of tumor neovascularization

Tumor neovascularization also plays an important role in malignant tumor growth and metastasis (Bao et al., 2012). Jatrorrhizine exerts antiproliferative effects in a dose-dependent manner with an IC<sub>50</sub> of 47.6  $\mu$ M, by overexpression of the cell-cycle-suppressive genes *p21* and *p27*, which resulted in human metastatic melanoma C8161 cell-cycle arrest at the G0/G1 transition (Liu et al., 2013). Meanwhile, jatrorrhizine showed antivascularogenic effects on C8161 cells with low toxicity, which is associated with impeding the expression of the gene for *VE-cadherin*, a key protein in tumor vasculogenic mimicry and angiogenesis (Liu et al., 2013). These results suggested that jatrorrhizine could be a potential new anti-melanoma drug candidate.

Similarly, columbamine could suppress human osteosarcoma U2OS cells proliferation *in vitro* with an IC<sub>50</sub> of 21.3  $\mu$ M, by inducing cell cycle arrest at the G2/M phase, which was associated with attenuating cell division protein kinase 6 (CDK6) gene expression and with diminishing STAT3 phosphorylation (Bao et al., 2012). In addition, columbamine could inhibit U2OS cell-mediated neovascularization by the down-regulation of matrix metalloproteinase MMP-2 expression and

reduction of cell migration, adhesion, and invasion, which suggested that columbamine could be a potential anti-osteosarcoma drug (Bao et al., 2012).

### Inhibition of tumor proliferation and invasion

Palmatine either in the purified form or a component of nexrutine (*Phellodendron amurense* bark extract) is a potent cytotoxic agent with growth and tumor invasion inhibitory properties (Hambright et al., 2015). It selectively inhibited the growth of prostate cancer cells (e.g. C4-2B, PC-3 and DU145 human prostate cancer cells) without significant effect on nontumorigenic prostate epithelial cells *in vitro*, for example, 10  $\mu$ g/mL palmatine was sufficient to inhibit the growth of DU145 cell lines by about 50%; at that dose PC-3 cell lines showed a 38% decrease in proliferation, which was measured using CellTiter 96 Aqueous One solution assay (Hambright et al., 2015). Further, treatment with palmatine significantly reduced promoter activity of both NF- $\kappa$ B (palmatine dose: 5  $\mu$ g/mL) and its target gene of the c-FLICE inhibitory protein (palmatine dose: 10  $\mu$ g/mL) in DU145 cell lines compared with control, which could potentially contribute to the inhibition of invasion in DU145 cells (Hambright et al., 2015).

### Antimicrobial activities

As two berberine's metabolites *in vivo*, palmatine and jatrorrhizine (200 and 400  $\mu$ g/mL), played antimicrobial roles by inhibition of the growth of bacteria *in vitro*, such as *Staphylococcus aureus* (Yu et al., 2007). Further results also indicated that palmatine and jatrorrhizine have shown relatively broad spectrum antimicrobial activities against animal pathogens *in vitro*, such as *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus lysodeikticus*, *Proteus vulgaris*, *Salmonella typhi* and *Escherichia coli*, with the minimum inhibitory concentration (MIC) values of 0.1–0.8 g/L and 0.1–0.6 g/L, respectively (Deng et al., 2012). Meanwhile, jatrorrhizine also exhibited the inhibitory activities against dermatophytes *in vitro* such as *Trichophyton rubrum* and *Microsporum canis* (MIC ranges from 62.5 to 125  $\mu$ g/mL), indicated jatrorrhizine could also be a potential antifungal agent (Vollekova et al., 2003).

### Anti-inflammatory effects

Inflammation is a common mechanism of many diseases. At the inflammatory foci, neutrophils could produce ROS, such as superoxide anion and nitric oxide (NO), which induces tissue damage. Lipopolysaccharide

(LPS)-induced inflammation was accompanied by the production of IL-6, NO, prostaglandin E2 (PGE2), the expression of cyclooxygenase-2 (COX-2) and iNOS mRNA, and the activation of NF- $\kappa$ B in murine macrophage RAW 264.7 cells (Mizokami et al., 2016). Jatrorrhizine, a major metabolite of berberine, was confirmed as an anti-inflammatory compound. In LPS-stimulated RAW 264.7 cells model, jatrorrhizine (100  $\mu$ g/mL) exhibited the anti-inflammatory effect, and its inhibitory activities against the expression of iNOS and COX-2 were 45% and 29%, respectively (Cho, 2011). On LPS-induced inflammation model in goat endometrial epithelial cells (gEECs) *in vitro*, palmatine treatment (80  $\mu$ g/mL) significantly decreased the production of pro-inflammatory factors, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, NO, and increased the production of anti-inflammatory factor IL-10 and pro-resolution mediator compared with the control group, which was measured with the enzyme linked immunosorbent assay (ELISA), quantitative RT-PCR and western blotting (Yan et al., 2017).

NF- $\kappa$ B has an established role in the regulation of innate immunity and inflammation (Gambhir et al., 2015). During inflammation, chemokines are a superfamily of small proteins, such as interleukin-8 and monocyte chemoattractant protein-1 (MCP-1) which are important in recruiting and activating leukocytes (Mishra et al., 2016). Berberrubine (1–25  $\mu$ M) dose dependently inhibited the increased levels of interleukin-8 and MCP-1 expression, protein secretion and NF- $\kappa$ B translocation in human retinal pigment epithelial (ARPE-19) cells stimulated with IL-1 $\beta$  or TNF- $\alpha$  (Cui et al., 2006). These findings indicated that berberrubine exhibited anti-inflammatory effects through similar mechanisms with berberine, which possibly benefited from its inhibition of interleukin-8 production (Zhou & Eshita, 2000).

## Discussion

Most of the herbal medicines are given via oral route in clinic. Only the components which are successfully absorbed into blood and maintain an effective concentration in target organs could be responsible for the therapeutic effects of herbal medicines. Those active ingredients may also yield a great deal of metabolites *in vivo* which could continue to exhibit the related biological activities. After absorption in small intestine, berberine is rapidly transformed into several kinds of main metabolites and has a low concentration in the plasma. Meanwhile, the organ concentration of berberine and its main metabolites was higher than that in the blood when orally administered (Tan et al., 2013). According to *in vivo* and *in vitro* pharmacological studies, the

major metabolites of berberine could also exhibit various biological activities, such as hypolipidemic effects and antitumor activities. Therefore, both berberine and its metabolites might be the active forms *in vivo* after berberine intake.

Based on the comprehensive studies for berberine metabolites in human and rats after oral administration, about 24 berberine-related metabolites have been identified. Among them, some metabolites were isolated from urine of humans or rats, and then their definite structures were elucidated by NMR and NOE spectroscopy (Qiu et al., 2008). Meanwhile, several metabolites of berberine *in vivo* have been confirmed by LC-MS/MS analysis (Yang et al., 2009; Zhang et al., 2008), which was a powerful method to identify the metabolites based on the MS data and LC retention times. However, the structures of the metabolites, which were deduced only using LC-MS/MS system, might be nebulous and impracticable, especially when isomeric metabolites were involved. For example, mono-glucuronide of demethyleneberberine was a main metabolite of berberine, but the hydroxyl group which is conjugated with the glucuronic acid is still unknown (Guo et al., 2011; Liu et al., 2009; Ma et al., 2009, 2013; Zuo et al., 2006).

Recent research has found that the gut microbiota could transform berberine after *in vitro* incubation (Feng et al., 2015; Wang et al., 2015b). These results were inconsistent with our findings (Qiu et al., 2008) and the previous report (Zuo et al., 2006), which berberine was relatively stable in the gastrointestinal tract. Gut microbiota might work as an "organ" to transform berberine into more efficient intestine-absorbable form such as dhBBR (Feng et al., 2015) or berberrubine (Li et al., 2014; Spinozzi et al., 2014), which could improve its oral bioavailability. Possibly, there are other explanations for the interaction between berberine and gut microbiota. We conjectured that the secondary metabolites of gut microbiota might also bring beneficial effects to improve the intestinal absorption of berberine, although to date, there is no available experimental data to confirm this conjecture. Meanwhile, berberine has inhibitory effects against microbial growth, which could decrease the number of intestinal bacteria, such as *Firmicutes* and *Bacteroidetes*, and reduce the diversity of intestinal microflora (Xie et al., 2011).

As for the toxicity of berberine, the route of administration is an important factor that affects the toxicity evaluation (Kheir et al., 2010). The median lethal dose (LD<sub>50</sub>) of berberine in mice after intravenous injection and intraperitoneal injection was calculated to be 9.03

and 57.6 mg/kg, respectively, but no LD<sub>50</sub> was found for the oral administration group (Kheir et al., 2010), which indicated that the acute toxicity of berberine was related to the type of administration route. Furthermore, it was found that 20.8 g berberine/kg of body weight is safe for oral administration in mice, and the safe dose for humans would be 2.97 g berberine/kg human body weight because the mice have a 7-fold metabolic rate per kg body weight than adult humans (Kheir et al., 2010). To date, no serious adverse effects have been reported for berberine via oral route in clinic, and it is safe in the majority of human subjects studied in short-term and chronically (Liu et al., 2016). However, some transient gastro-intestinal adverse effects have been observed after a high-dose administration of berberine (0.5 g, three times a day, in a 3-month trial) for the treatment of T2DM patients (Yin et al., 2008). As for the safety evaluation of berberine-related metabolites, a pilot study indicated that jatrorrhizine showed a very low toxicity after oral administration and its LD<sub>50</sub> in mice was about 5500 mg/kg which was significantly higher than that of berberine (LD<sub>50</sub>, 763 mg/kg) (Wu et al., 2014). However, the adverse effects of most berberine metabolites remain unclear.

## Conclusions

In summary, berberine is a safe and effective natural product, but the pharmacokinetic studies in rodents and humans have consistently concluded that berberine has poor systemic bioavailability after oral administration. Its poor absorption in the gut and rapid metabolism in the body are two major contributors to the low oral bioavailability. This work aimed to collect all data regarding metabolic profile of berberine. Many contributions to understand the metabolism of berberine have been made in the previous decade. O-demethylation, demethylenation, reduction, and subsequent conjugation with glucuronic acid and sulfuric acid are the major metabolic pathways for berberine *in vivo*. In recent years, much research has focused on the effects of metabolites described above, and several important metabolites could exhibit similar activities in comparison with berberine, which could be responsible for the therapeutic effects associated with berberine in clinical use. Nevertheless, the contribution of most metabolites of berberine is far from being clear, and further follow-up research is needed to explore this issue.

## Disclosure statement

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