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The natural anthraquinones from *Rheum palmatum* induced the metabolic disorder of melatonin by inhibiting human CYP and SULT enzymes

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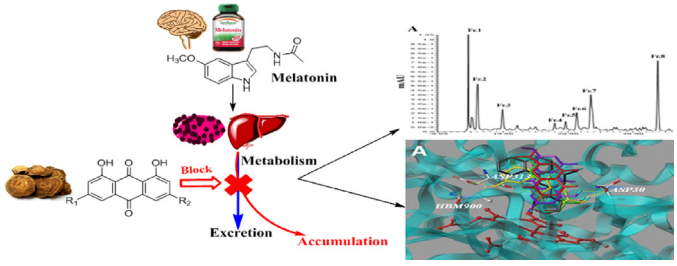
HIGHLIGHTS

- The herbs or foods containing abundant anthraquinones such as *R. palmatum* will cause a metabolic disorder of Mel, and should be avoided to combine application with Mel.
- Five anthraquinones including: aloeemodin (1), rhein (2), emodin (3), chrysophanol (4), and physcion (5) from *R. palmatum* exhibited significant inhibitory effect on the melatonin metabolism.
- The relationships between compounds' inhibition activities and their structures were also investigated using computational model to further explain the inhibition mechanism.

GRAPHICAL ABSTRACT



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ABSTRACT

Melatonin (Mel) as an endogenous hormone, has been widely used in clinic for multiple therapeutic purposes. Further, the natural anthraquinones were widespread in various plants including herbs, foods, and some flavoring agents. The present work aims to evaluate the metabolic disorder of Mel caused by

Abbreviations: Mel, Melatonin; 6-O-Mel, ,6-hydroxymelatonin; NAS, N-acetylserotonin; AFMK, N_1-Acetyl-N_2-formyl-5-methoxy-kinuramine; AMK, N-acetyl-5-methoxy-kinuramine; HDIs, herb–drug interactions; CYP450s, cytochromes P450; *R. palmatum*, *Rheum palmatum*; DDIs, drug–drug interactions; HLMs, human liver microsomes; G-6-P, glucose-6-phosphatedehydrogenas; $NADP^+$, β-nicotinamideadenine dinucleotidephosphate; DTT, dithiothreitol; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; $MgCl_2$, magnesiumchloride; HD-1, *Polygonum*bistorta L; HD-3, *Semen Cassiae* Obtusifoliae; HD-4, *Radix Polygoni* Multiflori; HD-5, *Radix Lithospermi*; HD-6, *Aloe*; HD-7, *Folium Sennae*; HD-8, *RhizomaPolygoni* Cuspidati; HD-9, *Radix Rubiae* Cordifoliae; HD-10, *Radix Salviae* Miltiorrhizae.

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various common herbs and further identify their underlying mechanism. More importantly, the relationships between inhibitory activity and their structures were also investigated. Our results demonstrate that some herbs containing anthraquinone derivatives exhibited strong inhibition on Mel metabolism. Additionally, five anthraquinones from *R. palmatum* could inhibit phase I and II metabolism of Mel with a mixed inhibition kinetic model based on the mechanism of inhibiting human CYP1A1, 1A2, and SULT1A1. At last, the influence of *R. palmatum* and its five major components on the Mel metabolism were verified in human primary hepatocytes. In conclusion, our studies elucidated that herbs or foods containing abundant anthraquinones such as *R. palmatum* will cause a metabolic disorder of Mel, and should be avoided to combined application with Mel *in clinic*.

1. Introduction

Mel is the principal endogenous hormone synthesized and secreted by the pineal gland during the dark period, and performs clock and calendar function in animals, plant and microbes (Bazrgar et al., 2015; Claustrat and Leston, 2015; Tan et al., 2015). Mel plays a vital role in the entrainment and regulation of circadian rhythms as a biological modulator of mood, sleep, immunity, reproduction, intestinal motility and sexual behavior (Arendt, 1998; Skwarlo-Sonta, 2002). Moreover, Mel is known to possess several properties of healthy aging, as a direct and indirect antioxidant, protectant and modulator of mitochondrial function, immune modulator and neuroprotectant (Hardeland et al., 2015; Hartter et al., 2000; Zetner et al., 2015). It tends to decrease in the course of senescence and be more strongly reduced in several neurodegenerative disorders, and in diseases related to insulin resistance such as diabetes type II (Miller et al., 2015; Polimeni et al., 2014). It has been reported that Mel deficiency was a key factor in this disorder. Nowadays, Mel as a health product is widely used in our daily life for the treatment of insomnia, especially for those who have difficulties in falling asleep (Hughes et al., 1998).

The clinical investigation indicated that Mel is metabolized in human, rapidly, with its half-life about 30–50 min in young adults. (Huuhka et al., 2006). And 6-hydroxylation is generally considered to be a major metabolic reaction in human, with *O*-demethylation representing a relatively minor pathway. Meantime, 6-hydroxymelatonin (6-*O*-Mel) and *N*-acetylserotonin (NAS) are further metabolized and excreted in urine as their sulfate conjugates (Hartter et al., 2001a). The previous study revealed that 6-hydroxylation of Mel is mainly mediated by human CYP1A1, CYP1A2, CYP1B1 and CYP2C19 (Facciola et al., 2001; Hartter et al., 2001b). However, in contrast to 6-hydroxylation, *O*-demethylation of Mel to form NAS is mediated by CYP2C19, with a minor contribution from CYP1A2 (Ma et al., 2005). After the phase I metabolism, 6-O-Mel and NAS are further metabolized by SULTs before excreted from urine, and SULT isoforms 1A1, 1A2, 1A3, 1B1 and 1E1 exhibit metabolic activities toward Mel (Riches et al., 2009; Tian et al., 2015). Additionally, Mel can be metabolized to N_1-Acetyl-N_2-formyl-5-methoxy-kinuramine (AFMK), and N-acetyl-5-methoxy-kinuramine (AMK) in the brains (Hardeland, 2010), however, AFMK and AMK are found to be trace or undetectable metabolites in human. Consequently, both CYPs and SULTs enzymes are responsible for the elimination of Mel.

As we all know, some herbal therapeutic efficacy is mild and broad, and the incidence of adverse reactions are relatively low in comparison with synthetic drugs (Elvin-Lewis, 2001). Therefore, herbs are increasingly employed world widely in alternative and complementary therapies. For example, the consumption of herbal products, commonly used as dietary supplements, natural health products, phytomedicines, and traditional medicines in United States of America, Canada, Europe or even in other developing countries, is becoming increasingly popular as alternative therapies to western medicine (Wei et al., 2013). Notably, they are frequently co-administered with therapeutic drugs, which dramatically raise the potential of clinical herb–drug interactions (HDIs). *Rheum palmatum (R. palmatum)* as a common medicinal and nutritional plant, gradually spread to India, Russia, Europe, and North America (Hsu et al., 2013; Zhang et al., 2013). It is widely used in clinic and daily life for various functions, such as purgative, choleretic, liver-protecting, antibacterial, anti-inflammatory, and anticarcinogenic effects. (Wang et al., 2011). Furthermore, anthraquinones are the most important type of components in *R. palmatum*, including aloeemodin, rhein, emodin, chrysophanol, physcion and their glucosides and so on (Sun et al., 2013; Zhang et al., 2013).

Interactions with enzyme catalyzing mediated reaction can occur through the inhibition and induction of cytochromes P450 (CYP450s), glucuronidation and sulfation enzymes. In addition, drug–drug interactions (DDIs) or herbal-drug interactions (HDIs) and toxicity mechanisms can be elucidated using CYP450s, glucuronidation and sulfation inhibition experiment (Brantley et al., 2014; Fong et al., 2012; Obach and Ryder, 2010). For example, some flavonoids can potently and selectively inhibit CYP1As isozymes (Zhai et al., 1998). Finasteride can potently, selectively, and competitively inhibit UGT1A4 mediated trifluoperazine-*N*-glucuronidation in human liver microsomes (HLMs) with a K_i value of 6.05 μM. And resveratrol can inhibit SULT1E1 activity in primary human mammary epithelial cells. All of these data suggest that the inhibition of herbals or natural products toward metabolic enzymes might be an important pathway for HDIs, which would induce the metabolic disorder of xenobiotics and endogenous substances in human (Papagiannidou et al., 2014).

The present study aims: 1) to investigate the metabolic disorder of Mel caused by some common herbs containing various anthraquinones; 2) to identify the major chemical constituents (anthraquinones) from *R. palmatum* that could result in potent metabolic inhibition of Mel and further explain the underlying mechanism; 3) to characterize the structure-dependent specific inhibition towards Mel metabolism, for explaining the potential inhibition toward Mel metabolism using the molecular docking.

2. Materials and methods

2.1. Materials

D-glucose-6-phosphate, glucose-6-phosphatedehydrogenas (G-6-P), β-nicotinamide adenine dinucleotide phosphate ($NADP^+$), dithiothreitol (DTT), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), magnesiumchloride ($MgCl_2$), 6-O-Mel, Mel were obtained from Sigma-Aldrich (St. Louis, MO). Authentic standards of aloeemodin (1), rhein (2), emodin (3), chrysophanol (4), physcion (5), shikonin (6), tanshinone II A (7) and purpurin (8) were purchased from Sichuan WeikeqiBiotechnology Company (Sichuan province, China). Pooled HLMs, and Pooled human liver cytosol (HLC) were all purchased from Rild Research Institute for Liver Diseases (Shanghai, China). Recombinant human CYP isoforms