Suppressed effects of *Phyllanthus urinaria* L. ethyl acetate extract on hepatitis B virus both in vitro and in vivo

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**Author contributions**

Anti-HBV assays in vitro and in vivo by Zhiling Chen; Study conception and drafting of the manuscript by Rui Zhang.

**Competing interests**

The authors declare no conflicts of interest.

**Acknowledgments**

This work was supported by Natural Science Foundation of Hainan Province (Grant No. 821QN0998); Key R&D Plan of Hainan Province (Grant No. ZDYF201813H2116); Postgraduate Innovation Project of Hainan Province (Grant No. Qyhyb2022-131, Qhsy2022-281); and supported by Hainan Province Clinical Medical Center.

**Peer review information**

Precision Medicine Research thanks Peng Zhan, Abbas Zabih and other anonymous reviewers for their contribution to the peer review of this paper.

**Abbreviations**

HBV, hepatitis B virus; TCM, traditional Chinese medicine; HBsAg, hepatitis B e antigen; HBcAg, hepatitis B surface antigen; P. urinaria, Phyllanthus urinaria L.; PUE, Phyllanthus urinaria L. extract; AF, aqueous fraction; EAF, ethyl acetate fraction; GA, gallic acid; CL, corilagin; ELSA, enzyme-linked immunosorbent assay; OD, optical density; HPLC, high performance liquid chromatograph; CCK-8, cell counting kit-8; ROS, reactive oxygen species; ω-3, superoxide anions; GSH, glutathione; SOD, superoxide dismutase; MOD, model group; ETC, entecavir solution group; NOR, non-diseased group; HYP, hydroxypropyl; ABC, ATP-binding cassette; GSH, glutathione; GSSG, glutathione disulfide.

**Citation**


**Abstract**

**Background:** *Phyllanthus urinaria* L. (*P. urinaria*) extract (PUE) has been used to inhibit hepatitis B virus (HBV). However, the underlying mechanism remains unclear. To investigate which PUE fractions and main components lead to against HBV and approach the relevant molecular mechanisms. **Methods:** *P. urinaria* was extracted with water, and then the decocation was extracted by petroleum ether, ethyl acetate, and n-butanol in turn. The HepG2.2.15 cell was treated with aqueous fraction, petroleum ether fraction, ethyl acetate fraction and n-butanol fraction, gallic acid (GA, C₇H₈O₆) and corilagin (CL, C₂₀H₂₄O₁₃), respectively. The medium was collected for hepatitis B surface antigen (HBsAg) and hepatitis B e antigen assays. Cell count-8 kit method was used to identify cell proliferation. Also, the levels of cellular oxygen consumption, reactive oxygen species, and reduced glutathione were detected. The HBV modeling mice were treated with ethyl acetate fraction, entecavir and physiological saline, respectively. The serum was collected for HBsAg and inflammatory cytokines assays. Liver tissue metabolites were screened by LC-MS/MS method. **Results:** The ethyl acetate fraction (EAF) of *P. urinaria* could significantly inhibit HBV secretion in HepG2.2.15 (*P* < 0.05). Furthermore, two main constitutes in ethyl acetate fraction, GA and CL, could significantly inhibit HBV secretion and reduced cell proliferation (*P* < 0.05). Also, GA and CL could increase cellular oxygen consumption, intracellular superoxide anions level, superoxide dismutase level and glutathione depletion. Compared with the Model group, EAF significantly decreased the expression levels of HBsAg, IL-1β, IFN-α (*P* < 0.05). LC-MS/MS analysis results showed that EAF dramatically up-regulate hydroxyproline, maltorirose, betaine and down-regulate glutathione disulfide, taurocholate, taurochenodeoxycholate (*P* < 0.05). Kyoto Encyclopedia of Genes and Genomes results show that the differential metabolites were mainly enriched in ATP-binding cassette transporters pathway. **Conclusions:** *P. urinaria* exhibits suppressed effects on HBV by modulating reactive oxygen species formation or metabolomics both in vitro and in vivo. These data indicate that *P. urinaria* may be an alternative therapeutic agent for the treatment of HBV-related hepatitis.

**Keywords:** hepatitis B virus; *Phyllanthus urinaria* L.; fractions; reactive oxygen species formation; metabolomics
**Introduction**

Hepatitis B virus (HBV) infection is a major health challenge worldwide. Chronic hepatitis B patients have a higher risk of emergence of cirrhosis and strongly associate with development of hepatocellular carcinoma, which is responsible for about 54% of global liver cancers [1]. Hepatocellular carcinoma is one of the major’s health risks worldwide and is one of the top three causes of cancer-related deaths worldwide [2]. HBV shows obvious drug resistance after long-term therapy and side effects for treatments developed and approved for chronic hepatitis B (e.g., interferon and nucleoside/nucleotide analogs) [3], therefore, continuous development of agents for the treatment of HBV infection is urgently needed.

Traditional Chinese medicine (TCM) became alternative candidates for treating hepatitis B in China and some other countries [4]. Over the past few years, various Chinese herbal formulas and single herb have been garners increasing attention for its effectiveness and beneficial contribution on HBV. Furthermore, various Chinese medicine preparations and related active compounds were discovered and played pivotal roles in anti-HBV drug development process [5]. TCM exhibit useful anti-HBV activities with enhanced hepatic function [6, 7], inhibited HBV DNA replication and reduced hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg) expression [8].

Phyllanthus urinaria L. (P. urinaria) has long been used to promote health in TCM and used as an immune system stimulator [9, 10]. This herb is also used for protective effects against liver disorders [11, 12]. Studies reveal that P. urinaria extract (PUE) has inhibition effect on HBV replication and expression in HBV transfected cell lines or duck HBV model [13, 14]. Jung, et al. show that PUE has antiviral activity by inducing the genes expression, such as IFN-β, COX-2, and IL-6 [15]. Antiviral activity against lamivudine resistant mutants was induced by inducing expression of IFN-β, COX-2 and IL-6. However, it is still mostly unknown the underlying mechanism of anti-HBV activities of PUE. Therefore, we aimed to evaluate the anti-HBV capacity of PUE and its activity components and explore related molecular mechanisms both in vitro and in vivo.

**Methods and materials**

**Preparation of plant extract**

P. urinaria was purchased from Tongrentang pharmacy (Haikou, China). Dried leaf of P. urinaria was crushed and then extracted with water to gain aqueous fraction (AF). AF was then extracted by petroleum ether, ethyl acetate, and n-butanol in turn, and gained petroleum ether fraction, ethyl acetate fraction (EAF) and n-butanol fraction. Obtained individual extracts were dried to powder, then re-dissolved in dimethyl sulfoxide as stock solution (200 mg/mL) and stored at -20 °C until used.

**Cell culture**

HepG2.2.15 cell was cultured in DMEM medium (Gibco, Thermo Fisher Scientific Co., Ltd., Waltham, MA, USA) with 10% fetal bovine serum and 1% P/S under a humidified atmosphere at 37 °C/5% CO₂. After cultured 24 h, the extracts, gallic acid (GA, C₇H₆O₅) and corilagin (CL, C₂₀H₁₄O₁₀), were diluted to proper concentrations and added into the medium.

**Quantification of HBsAg and HBeAg**

The level of HBsAg and HBeAg were measured by using enzyme-linked immunosorbent assay (ELISA) kits (Kehua Bio-engineering Co., Ltd., Shanghai, China). In brief, the culture media was added into plates coated with antibody and incubated at 37 °C for 30 min. Then washed three times and incubated with substrate solution at 37 °C for 15 min. The optical density (OD)450 values were determined.

**High performance liquid chromatograph (HPLC) analysis**

The ethyl acetate fraction component was detected on Agilent 1220 Infinity II (Agilent technologies Inc., Santa Clara, CA, USA). The chromatographic conditions are Diamonsil C18 (250 mm × 4.6 mm, 5 μm), chromatographic column mobile phase: acetonitrile –0.2% glacial acetic acid solution, gradient elution: 0–10 min (acetonitrile: 5%–12%), 10–25 min (acetonitrile: 12%–16%), 25–40 min (acetonitrile: 16%–17%), 40–50 min (acetonitrile: 17%); column temperature: 30 °C; flow rate: 1 mL/min; detection wavelength: 270 nm.

**Cell proliferation assay**

The cell proliferation was measured by using cell counting kit-8 (CCK-8) kit (Dojindo Laboratories, Shanghai, China). Briefly, 103 cells the cells were planted into the well of 96-well plates and grewed for 48 h. Then 10 μL CCK-8 was added into the well for 1 hour and the OD450 values were determined.

**Oxygen consumption assay**

All group of samples were dissociated and washed with phosphate buffered saline. Cells re-suspended to 1x10³ cells/mL in DMEM medium. Oxygen consumption was measured by using Clark-type electrode (Hansatech Instruments Co., Ltd., Norfolk, UK).

**Intracellular reactive oxygen species (ROS) measurement**

Intracellular superoxide anions (O₂⁻) was assessed by the dihydroethidium fluorescence probe. After applying 10 μM dihydroethidium (Thermo Fisher Scientific Co., Ltd., Waltham, MA, USA), the cells were incubated at 37 °C for 30 min in a dark chamber.

**Measurement of reduced glutathione (GSH) and increased superoxide dismutase (total SOD, CuZn-SOD)**

The level of GSH, total SOD and CuZn-SOD was detected by using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer’s instructions.

**Animals**

Male BALB/cJGpt mice at the age about 6 weeks were obtained from Jiangsu GemPharmatech Co., Ltd. (Nanjing, China), and were kept in specific pathogen-free room. The experiments were conducted obeying the Chinese Guidelines of Animal Care and Welfare and approved by the Ethics Committee of Hainan Medical University (No. HYLL-2022-068).

**Animal model and drug gavage**

Dilute 10 μg plasmid pcDNA3.1 (+) /HBV-DNA according to 0.1 mL/g of mouse body weight with physiological saline, and then inject the solution into the tail vein of each mouse within 10 seconds. Mice were injected with physiological saline as the control group. After 24 hours, collect samples of mice serum and measure alanine aminotransferase (ALT) and HBsAg levels using an ELISA kit (China Kehua Biotechnology Co., Ltd., Shanghai, China). The model mice were selected and divided into P. urinaria EAF group, model group (MOD) and entecavir solution group (ETC), with 8 mice in each group. Select 8 mice from the control group as the non-diseased group (NOR). The weight of all animals is approximately. EAF and ETC mouse groups were orally administered with 0.2 mL of reagent. We calculated the equivalent dose of human and mice. The EAF mice group was given 2.0 g/kg/d EAF for 10 days, the ETC mice group was given 3.2 mg/kg/d entecavir, and the other group was given distilled water at the same volume.

**Determination of hepatitis B biomarkers and inflammatory cytokines**

On the 10th day, all animals were euthanized, and then blood was taken from the posterior orbital vascular plexus of mice using sterile Eppendorf tubes. The serum was separated by centrifugation at 2000 g for 10 minutes. Detection of HBsAg, IL-1β and IFN-α in serum was performed through ELISA assay, and the protocol was the same as the
Applying ultraperformance liquid chromatography quadrupole-time-of-flight hybrid mass spectrometry method for liver tissue metabolomics analysis
This experiment relies on the implementation of The Applied Protein Technology Co., Ltd. (Shanghai, China). The protocol, final processing and result analysis of the dataset were as described earlier [16].

Statistics analysis
All data were presented as the mean ± standard deviation (SD) (N = 8). Comparisons between groups were made with two-way analysis of variance, using GraphPad Prism (GraphPad Software, San Diego, CA, USA). The difference was considered statistically significant when the P-value was < 0.05.

Results
The effect of various extract/fractions on HBsAg and HBeAg secretion
We determined the effects of the PUE on HBsAg and HBeAg secretion by HepG2.2.15 cells. Of note, the EAF was more efficient in inhibiting the HBsAg and HBeAg secretion, while compared to other extracts (Figure 1). After 48 h of EAF (50 and 100 mg/L) treatment, the secretion of HBsAg was reduced by 32.81 ± 2.96% (P < 0.05) and 51.38 ± 4.36% (P < 0.05) and that of HBeAg by 33.75 ± 3.12% (P < 0.05) and 55.64 ± 4.83% (P < 0.05), respectively.

The composition of the P. urinaria EAF

The amounts of the two major components of P. urinaria EAF (gallic acid and corilagin) were determined by HPLC. Gallic acid (26.8%) was detected as a major peak. Corilagin (8.15%) was also identified, but the peak was small (Supplementary Figure S1).

The effect of GA and CL on HBsAg and HBeAg secretion
Furthermore, we observed that GA and CL inhibited HBsAg and HBeAg secretion in a concentration-dependent manner (Figure 2). After 48 h of GA treatment (25 and 100 mg/L), the secretion of HBsAg was reduced by 31.32 ± 2.24% (P < 0.05) and 48.19 ± 3.64% (P < 0.05) and that of HBeAg by 41.18 ± 3.99% (P < 0.05) and 62.28 ± 4.47% (P < 0.05), respectively. Meanwhile, after 48 h of CL treatment (25 and 100 mg/L), the secretion of HBsAg was reduced by 65.04 ± 5.57% (P < 0.05) and 81.11 ± 6.72% (P < 0.05) and that of HBeAg by 63.71 ± 6.14% (P < 0.05) and 83.69 ± 7.37% (P < 0.05), respectively.

The effect of GA and CL on cell proliferation
To determine whether the anti-HBV effects of the EAF were due to cytotoxicity, the effects of GA and CL on HepG2.2.15 cell proliferation were assessed by the CCK-8 assay (Figure 3). The results showed that GA and CL inhibited the cell proliferation in a concentration dependent manner. After 48 h of GA treatment (25 and 100 mg/L), the cell viability was significantly reduced by 52.71 ± 5.06% (P < 0.05) and 83.62 ± 5.33% (P < 0.05), respectively. After 48 h of CL treatment (25 and 100 mg/L), the cell viability was significantly reduced by 73.60 ± 6.23% (P < 0.05) and 93.11 ± 7.25% (P < 0.05), respectively.

Figure 1 Different fractions of PUE effect on HBV secretion. We detect (a) HBsAg and (b) HBeAg by ELISA. Data represent the mean ± SD (n = 8). * P < 0.05 for significant difference of various fractions treated group compared with untreated group. HBV, hepatitis B virus; HBsAg, hepatitis B e antigen; HBeAg, hepatitis B surface antigen; PUE, Phyllanthus urinaria L. extract; AF, aqueous fraction; EAF, ethyl acetate fraction; PEF, petroleum ether fraction; BF, n-butanol fraction; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation.

Figure 2 The effect of GA and CL on HBV secretion. GA and CL could significantly reduce (a) HBsAg and (b) HBeAg secretion. Data represent the mean ± SD (n = 8). * P < 0.05 for significant difference of GA/CL treated group compared with untreated group. HBV, hepatitis B virus; HBsAg, hepatitis B e antigen; HBeAg, hepatitis B surface antigen; GA, gallic acid; CL, corilagin; SD, standard deviation.
The effect of GA and CL on oxidative stress
To determine whether GA and CL reduced proliferation was involved in oxidative stress, the level of oxygen consumption, $O_2^\cdot$ production, total SOD and CuZn-SOD were detected. After 48 h of GA treatment (25 and 100 mg/L), the cell oxygen consumption was significantly increased by $26.24 \pm 1.16\%$ ($P < 0.05$) and $49.82 \pm 3.16\%$ ($P < 0.05$), respectively. After 48 h of CL treatment (25 and 100 mg/L), the cell oxygen consumption was significantly increased by $28.26 \pm 2.28\%$ ($P < 0.05$) and $32.53 \pm 2.94\%$ ($P < 0.05$), respectively. The results showed that GA and CL enhanced the cell’s oxygen consumption (Figure 4a).

As shown in Figure 4b, GA and CL enhanced the intracellular $O_2^\cdot$ production. After 48 h of GA treatment (10, 25 and 100 mg/L), the intracellular $O_2^\cdot$ production was significantly increased $141.64\%$ ($P < 0.05$), $124.29\%$ ($P < 0.05$) and $192.38\%$ ($P < 0.05$), respectively. After 48 h of CL treatment (10, 25 and 100 mg/L), the intracellular $O_2^\cdot$ production was significantly increased $156.85\%$ ($P < 0.05$), $127.23\%$ ($P < 0.05$) and $123.17\%$ ($P < 0.05$), respectively. As shown in Figure 4c, GA and CL decreased the intracellular GSH level. After 48 h of GA treatment (10, 25 and 100 mg/L), the GSH level was significantly decreased $28.94\%$ ($P < 0.05$), $66.31\%$ ($P < 0.05$) and $76.64\%$ ($P < 0.05$), respectively. After 48 h of CL treatment (10, 25 and 100 mg/L), the GSH level was significantly decreased $34.24\%$ ($P < 0.05$), $45.43\%$ ($P < 0.05$) and $77.72\%$ ($P < 0.05$), respectively.

As shown in Figure 4d and 4e, GA and CL increased the total SOD and CuZn-SOD level. After 48 h of GA treatment (10, 25 and 100 mg/L), the total SOD level was significantly increased $122.41\%$ ($P < 0.05$), $127.23\%$ ($P < 0.05$) and $123.17\%$ ($P < 0.05$), respectively. The CuZn-SOD level was significantly increased $49.71\%$ ($P < 0.05$), $123.18\%$ ($P < 0.05$) and $48.64\%$ ($P < 0.05$), respectively. After 48 h of CL treatment (10, 25 and 100 mg/L), the total SOD level was significantly increased $269.83\%$ ($P < 0.05$), $204.34\%$ ($P < 0.05$) and $264.57\%$ ($P < 0.05$), respectively. The CuZn-SOD level was significantly increased $44.74\%$ ($P < 0.05$), $73.21\%$ ($P < 0.05$) and $28.66\%$ ($P < 0.05$), respectively.

![Figure 3 The effect of GA and CL on cell proliferation.](https://www.tmrjournals.com/pmr)

![Figure 4 The effect of GA and CL on oxidative stress.](https://www.tmrjournals.com/pmr)

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EAF reduces HBV clinical symptoms
To investigate the effects of the EAF on anti-HBV effect in vivo, we firstly constructed an HBV animal model, as shown in Figure 5, the levels of alanine aminotransferase activity and HBsAg in serum of the modeling group dramatically increased when compared with the control group. It demonstrated HBV model was successfully induced. Then, HBsAg, IL-1β, and IFN-α levels in the serum were measured by ELISA, the results show that EAF treated group exhibit a significant decline of HBsAg (P < 0.05), IL-1β (P < 0.05), IFN-α (P < 0.05) levels compared with modeling group (Figure 6a and 6b).

EAF changes liver tissue metabolites
The results in Supplementary Figure S2 show that the response intensity and retention time of each chromatographic peak basically overlap, indicating that the changes caused by instrument errors are relatively small throughout the entire experimental process.

Based on the relative abundance of positive and negative ion metabolites, PCA, PLSDA, and OPLS-DA showed that the model group mice exhibited a distinct microbial community composition compared to other groups (Supplementary Figure S3a–3f).

The volcano map shows the differential metabolites of positive and negative ions between the EAF and MOD groups, with significant differential metabolites appearing in pink (Figures 7a and 7b). We applied variable influence on projection (VIP) values >1 and P < 0.05 analysis at the univariate level to the student t-test to measure the significance of each metabolite and screen out 13 upregulated and 9 downregulated differential metabolites (Table 1). As the results show that the EAF of P. urinaria treatment-modulating liver metabolites such as up-regulating hydroxyproline (HYP), maltotriose, betaine and down-regulating glutathione disulfide, taurocholate, taurochenodeoxycholate (P < 0.05).

As shown in Figure 8, the differential metabolites were analyzed by Kyoto Encyclopedia of Genes and Genomes and enriched pathways. $-\log_{10}(P$ value) was represent with color from red to green, and the number of metabolites was present according to the size ratio listed. As the results show that the differential metabolites were mainly enriched in ATP-binding cassette (ABC) transporters pathway.

Discussion

The P. urinaria has long been used as an anti-HBV herbal medicine. In the present study, we showed that EAF has an effective antiviral capacity, and the main component of EAF, GA and CL, could effectively against HBV. We also showed that GA and CL have vital role in inhibition of cell proliferation. Furthermore, we analyzed the intracellular oxidative stress-related molecules, and found that oxygen consumption, $O_2^-$ and SOD production was increased, while GSH level was decreased. Moreover, we displayed that EAF has a suppressed effects on HBV symptoms in mice, and also modulate liver tissue metabolomics.

Multiple components with medicinal properties such as flavonoids, carboxylic acids, tannins, and lignans, have been identified from P. urinaria [17]. Studies showed that decoction of P. urinaria could exert distinctive anti-HBV effects in HBV transfected cell lines in vitro [14, 15]. Shin MS, et al. showed that a flavonoid molecule isolated from P. urinaria has a unique anti-HBV function [18]. Consist of these previous researches, we found that AF has an inhibitory role in the HBV metabolism.

![Figure 5 Induction of HBV mouse model](image-url)

*Figure 5 Induction of HBV mouse model. We detect ALT and HBsAg by ELISA. Data represents the mean ± SD (n = 8), *P < 0.05 for significant difference of Modeling group compared with the Control group. HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation.*

![Figure 6 The effect of EAF on HBsAg, IL-1β, and IFN-α](image-url)

*Figure 6 The effect of EAF on HBsAg, IL-1β, and IFN-α. We detect (a) HBsAg, (b) IL-1β and IFN-α by ELISA. Data represents the mean ± SD (n = 8). *P < 0.05 for significant difference of various reagent treated group compared with model group. EAF, ethyl acetate fraction; HBsAg, hepatitis B surface antigen; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; MOD, model group; ETC, entecavir solution group; NOR, non-diseased group.*

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Figure 7 Effects of EAF for HBV mouse model on the liver tissue metabolites. The difference in metabolites between the EAF treatment group (labeled with YXZ) and the model group (labeled with Mod) was shown in the (a, b) volcanic map. The pink metabolite is considered statistically significant. EAF, ethyl acetate fraction; HBV, hepatitis B virus; MOD, model group; YXZ, Yexiazhu, Phyllanthus urinaria L.

Table 1 Differential metabolites

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VIP, variable influence on projection; POS, positive; NEG, negative.
secretion. Furthermore, EAF showed better anti-HBV capacity, while petroleum ether fraction and n-butanol fraction had less or no obvious effect. Therefore, we demonstrated that the main anti-HBV constituents were present in EAF.

The HPLC assay demonstrated that the GA and CL were two main constituents in EAF. In this study, both GA and CL exhibit anti-HBV activity. GA is a natural phenolic compound that possesses antiviral activities [19–21]. GA could down-regulate the expression levels of Nf-κB/HCV protein and HCV-RNA [22], and inhibit HCV entry [23]. Meanwhile, CL also possesses antiviral activities [24, 25]. Notably, the CL had better anti-HBV activity than that of AF and EAF. 25 mg/L CL could markedly reduce HBsAg and HBeAg secretion.

Studies showed that GA could increase ROS levels and induced GSH depletion [26–28]. This study confirms that GA and CL would induce GSH depletion. Interestingly, GA and CL increased cells oxygen consumption, which suggest more O2 participated in O2·- production. It is reported that SOD could transform O2·- to H2O2, therefore, we further detected the activity of SOD, and found that the activity of SOD, including total SOD and CuZn-SOD, was increased (Figure 4d and 4e). These results indicated that the cell enhanced its anti-oxidative enzyme activity to reduce over-produced O2·-

ROS act as secondary messengers mediates various cellular processes such as cellular proliferation, cell survival, and inflammation [29]. Studies showed that over-production ROS could inhibit cell proliferation [30, 31]. In the present study, GA and CL reduced HepG2.2.15 cell proliferation, which would be helpful to kill the cells infected by HBV.

To further determine whether P. urinaria remains in effect in vivo and approach the relevant molecular mechanisms, we observed its anti-infection efficacy and impact of changing the liver metabolites in HBV mouse model. The EAF of P. urinaria directly hampered the production of HBsAg, IL-1β, IFN-α in serum thus reducing the loss of HBV to the body. Additionally, we monitored the health conditions and body weight of mice, and P. urinaria is proved to be a biosafety medicinal plant that can be used persistently.

Investigating the metabolomics may provide a new mean to investigate the process and action of mechanism of P. urinaria on interfering HBV infection. As the results show that EAF treatment modulating liver metabolites such as up-regulating HYP, maltooltriose, betaine and down-regulating glutathione disulfide, taurocholate, taurochenodeoxycholate. And the differential metabolites were mainly enriched in of ABC transporters pathway.

HYP is one of the main components of collagen in the body, and its content in normal collagen is about 13.4%. The amount of HYP in tissues is an important indicator of the metabolic capacity [32]. Betaine is a natural substance that regulates the internal environment of the organism and enhances its activity [33]. Betaine regulates the metabolism of nutrients in the body mainly in the form of trans-methyl donors in animals and maintains the normal functioning and energy expenditure of the body [34]. And maltooltriose is also a critical energy source for tissues. P. urinaria may be able to defend against viral attack and effectively eliminate pathogens by elevating the levels of these beneficial metabolites in the liver.

Oxidative stress is the excessive production of free radicals in the body, such as oxygen radicals, which can disrupt the balance of oxidative and antioxidiant activity in the body, leading to cellular damage. The glutathione (GSH)/glutathione disulfide (GSSG) ratio was found to be a marker for early hepatotoxicity [35]. Zhang, et al. also validated the GSH/GSSG ratio as a biomarker in a study of diseases causing oxidative stress [36]. Our results in vitro and in vivo confirm that EAF or its major components significantly reduce GSH or GSSH levels, showing effective completion of injury via ROS formation and simultaneous reduction of excessive injury by controlling the GSH/GSSH ratio.

Taurocholate and taurochenodeoxycholate are the main component of taurocholic acid. Luo, et al. evaluated the value of three bile acids, cholic acid, glycocholic acid and taurocholic acid, as potential biomarkers of liver injury and found that all they were significantly elevated during hepatocyte necrosis [37]. Therefore, taurocholic acid can be used to evaluate liver injury. Our data show that P. urinaria is
effective in reducing liver damage and exerts excellent hepatoprotective effects while being antiviral.

Transporters are involved in the transmembrane transport of drugs and endogenous substances, together with metabolic enzymes. They are responsible for the in vivo pharmacokinetic processes of drug absorption, distribution, metabolism and excretion [38]. The efflux transporter mainly mediates the extracellular excretion of substances, and this type of transporter generally belongs to the ABC transporter family. Liver expresses a variety of transporters [39]. Changes in the expression of ABC transporters pathway under disease or drug disposition conditions could serve as signals for disease progression or targets for liver disease therapy [40]. This also implies that P. urinaria affect the ABC transporters pathway for better antiviral effects.

Conclusion

Summarizing the main research findings, P. urinaria has anti-HBV capacity in HBV transfected cell lines in vitro and in HBV mouse model in vivo. Especially, EAF of P. urinaria has better anti-HBV capacity than of the other fraction. Both two main constituents of EAF, GA and CL, could enhance O3 production which is the main intracellular ROS source. The over-production of intracellular ROS would reduce cell proliferation, thus reduce HBV secretion. Also, EAF of P. urinaria may be able to anti HBV attack by elevating beneficial metabolites, controlling the GSH/GSSH ratio, reducing liver damage and affecting the ABC transporters pathway for better antiviral effects. Therefore, we suggest the P. urinaria could serve as main adjuvant therapy agent in HBV-related hepatitis.

References

Available at: http://doi.org/10.1007/s12035-014-8947-7


