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LETTER TO THE EDITOR

Veronica anagallis-aquatica L. iridoid glycosides alleviate heart failure *via* metabolites homoveratrumic acid and 2-hydroxy-3,4-dimethoxybenzoic acid mediated by the gut microbiota

KEY WORDS

Iridoid glycosides;
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Cardiac hypertrophy;
HDAC2

To the Editor

Heart failure (HF) is a multifactorial-induced chronic cardiovascular disease characterized by high mortality rates and poor patient prognosis. While current therapeutic interventions may relieve symptoms and enhance survival rates, the 5-year survival rate for patients remains below 50%[1]. This limitation may stem from the fact that the underlying pathophysiological mechanisms of HF have not yet been fully elucidated. Currently, clinical drug therapy primarily involves polypharmacy (quadruple therapy).

The complex regulatory mechanisms required for major chronic diseases like HF are well-aligned with the multi-component and multi-target nature of Chinese herbs. Therefore, the exploration and development of Chinese herbs could emerge as a focal point in the quest for innovative therapeutic strategies for HF. In this study, the ethanol extract of *Veronica anagallis-aquatica* L., identified from an in-house library of 828 Chinese herbs, demonstrated significant anti-HF potential. Further component analysis revealed that a cluster of iridoid glycoside analogs (referred to as **HL0877i**) act as the principal anti-HF active ingredient in *Veronica anagallis-aquatica* L.

Subsequent analysis of the 16S rDNA gene sequencing data indicated that **HL0877i** induced a remodeling of the gut microbiota. Additionally, fecal microbiota transplantation (FMT) assays confirmed that **HL0877i** alleviated HF in a gut microbe-dependent manner, highlighting the gut microbiota's potential role in mediating the anti-HF effects of **HL0877i**. This finding is consistent with a growing body of research that highlights the potential of various Chinese herbs to influence the gut microbiota positively. However, there is a lack of research elucidating the specific compounds that are crucial in this process and the underlying mechanisms of treatment. Herein, we discovered that two key active metabolites, homoveratrumic acid (**HVA**) and 2-hydroxy-3,4-dimethoxybenzoic acid (**2OH−VA**), were increased in the intestinal environment regulated by **HL0877i**. Mechanistically, **HVA** and **2OH−VA** attenuate cardiac dysfunction and hypertrophy by suppressing the HDAC2−KLF4/INPP5F−AKT−GSK3β signaling pathway. In conclusion, the present study provides new insight into the mechanism underlying the anti-HF efficacy of iridoid glycosides originating from the Chinese herb *Veronica anagallis-aquatica* L. by modulating the gut microbiota and influencing the production of two metabolites, **HVA** and **2OH−VA**, providing substantial support for anti-HF therapy.

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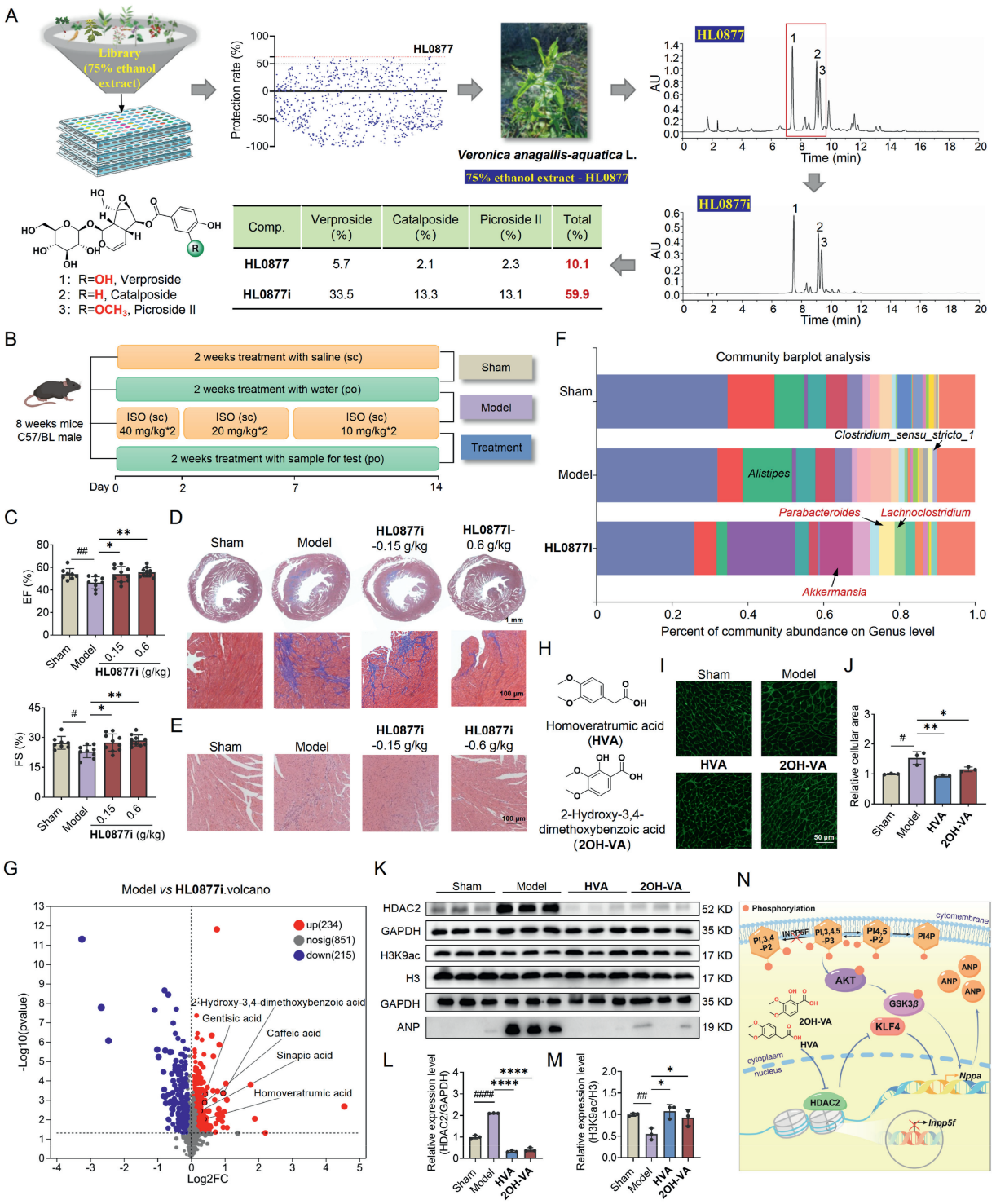

Figure 1 Iridoid glycosides HL0877i originating from the Chinese herb *Veronica anagallis-aquatica* L. alleviate HF, which was driven by gut microbiota and metabolites (HVA and 2OH−VA). (A) Screening, active ingredient identification, and enrichment of HL0877 derived from *Veronica anagallis-aquatica* L. (B) Schematic representation of the administration of different groups. (C) HL0877i enhanced the cardiac function of ISO-induced HF mice (n = 10). (D) HL0877i decreased myocardial fibrosis evaluated by Masson staining, scale bar = 1 mm or 100 μm. (E) HE staining revealed that HL0877i reduced myocardial damage, scale bar = 100 μm. (F) The community of the gut microbiota at the genus level. The representative microbes with augmented abundance in the model group (black font) and HL0877i group (red font) were indicated (n = 10). (G) Volcano plots of fecal metabolites across the model and HL0877i group. A series of aromatic acid compounds were significantly upregulated in the HL0877i group (n = 6). (H) Chemical structures of representative active metabolites, HVA and 2OH−VA. (I) Representative WGA images show the cellular area of heart sections, scale bar = 50 μm. (J) Statistical analysis of the cellular area, HVA, and 2OH−VA mitigated cardiac hypertrophy. Each dot represents a mouse, and more than five regions were quantified for each mouse. (K) HVA and 2OH−VA decreased the

1. Iridoid glycosides (HL0877i) concentrated from *Veronica anagallis-aquatica* L. shows potential as an anti-HF therapeutic agent

First, we screened 828 ethanol extracts of particular medicinal plants from an in-house library, which originated from endemic plants in the western region of Yunnan Province, China, to discover valuable Chinese herbs with cardiomyocyte protective effects. Among them, *Veronica anagallis-aquatica* L. ethanol extract was identified as the most active extract (**HL0877**). After confirming the anti-HF activity of **HL0877** *in vivo* and *in vitro* (Supporting Information Fig. S1), we further explored what components play a major role in the activity. The reported components of *Veronica anagallis-aquatica* L. mainly include iridoid glycosides, phenylethanoid glycosides, flavonoids, and steroids[2]. Herein, iridoid glycosides (**HL0877i**) were extracted and purified *via* preparative liquid chromatography. Verproside, catalposide, and picroside II were the predominant components in this novel enriched fraction, with their combined content increased from 10.1% to 59.9% (*w*/*w*; Fig. 1A). These three components protect cardiomyocytes from oxygen-glucose deprivation (OGD, Supporting Information Fig. S2). Additionally, 18 other components were inferred, mainly flavonoids, organooxygen compounds, cinnamic acids and derivatives, benzene and substituted derivatives, prenol lipids, and others (Supporting Information Fig. S3 and Table S1).

Subsequently, the cytoprotective effects of **HL0877i** were evaluated in the OGD injury model. We demonstrated that **HL0877i** has a comparable ability to **HL0877** in enhancing cell viability and in reducing levels of reactive oxygen species and lactate dehydrogenase (Supporting Information Fig. S4A−S4C). Moreover, compared with **HL0877**, **HL0877i** had a superior anti-HF effect *in vivo* (Fig. 1B−E and Fig. S4D). **HL0877i** administration at 150 mg/kg significantly mitigated the decline in ejection fraction (EF) and fractional shortening (FS) in isoprenaline (ISO)-induced HF mice. At 600 mg/kg, it further alleviated myocardial damage and fibrosis, comparable to 1.2 g/kg of **HL0877**, suggesting the importance of iridoid glycosides in maintaining the cardioprotective effects. Additionally, a two-week repeat-dose toxicity study was performed to assess the safety profile of **HL0877i**. The results indicated no cumulative toxicity following sustained oral administration of **HL0877i** (6 g/kg, Supporting Information Fig. S5). In conclusion, **HL0877i** effectively enriched the active constituents and demonstrated a favorable safety profile.

2. HL0877i exerted a protective effect against HF by reshaping the gut microbiome of mice

Several cohort studies have reported that the changes in the gut microbial community are associated with poor prognosis of HF patients[3]. These findings offer a novel perspective to elucidate the therapeutic mechanisms of traditional Chinese herbs, which involve the modulation of gut microbiota as a therapy strategy to treat HF. In our study, 16S rDNA sequencing revealed that **HL0877i** not only decreased community diversity and richness (Supporting Information Fig. S6A−S6D) but also altered the relative abundance of specific gut microbes at various taxonomic levels (Fig. S6E and S6F and Fig. 1F). At the genus level, as shown in Fig. 1F and detailed in Table S2, **HL0877i** increased the relative abundance of beneficial bacterial genera, including *Akkermansia*, *Parabacteroides*, and *Lachnoclostridium*. It reversed alterations caused by ISO-induced injury, characterized by an increase in *Alistipes* and *Clostridium_sensu_stricto_1*. In subsequent FMT trials, changes in these five strains were again observed (Table S3), indicating that they may represent characteristic strains associated with disease status.

An FMT experiment was conducted to ascertain the importance of reshaping the gut microbiota through **HL0877i** in treating HF. Recipient mice were pretreated with an antibiotic cocktail and then orally administered a fecal bacterial suspension from **HL0877i**-treated donor mice daily (Supporting Information Fig. S7A). After FMT treatment, the EF and FS of recipient mice were significantly enhanced (Fig. S7B), while the serum levels of BNP and inflammatory cytokines, TNF-α and IL-6, were also significantly decreased (Fig. S7C and S7D). Additionally, the extent of myocardial damage, fibrosis, and hypertrophy was alleviated (Fig. S7E−S7H) compared to the model group. These findings indicated that the mice were effectively treated for HF undergoing FMT. Then, fecal samples from the four groups of mice were collected for 16S rDNA sequencing, which confirmed that the recipient and donor groups shared a high degree of similarity (Supporting Information Fig. S8), with no significant differences observed in the Shannon or ACE indices and bacterial composition. Based on these findings, the transplanted gut microbiota appears to have been successfully colonized in the recipient mice, exhibiting an anti-HF effect.

3. Supplementation of the key metabolite, either HVA or 2OH−VA, ameliorates cardiac hypertrophy to improve HF

The gut microbiota regulates the synthesis and absorption of microbial metabolites. Consequently, we hypothesized that metabolic changes might underlie the effects of **HL0877i** on HF. First, significant variations in metabolite profiles were observed by the untargeted metabolomics analysis on fecal samples (Supporting Information Fig. S9A). Focusing on the differential metabolites between the groups, we identified 449 metabolites, of which 364 correspond to HMDB entries, in comparing the model and **HL0877i** groups. Of these, 63.19% were classified as lipids and lipid-like molecules (Fig. S9B−S9D). We noticed that **HL0877i** upregulated the levels of **2OH−VA** (Fig. S9B), a phenolic acid compound. Furthermore, the volcano plots clearly depicted that the abundances of five metabolites (**2OH−VA**, gentisic acid, caffeic acid, sinapic acid, and **HVA**) that belong to phenolic acids and derivatives were significantly upregulated in the **HL0877i** group (Fig. 1G).

Subsequently, **2OH−VA**, which showed the most significant upregulation in abundance (Supporting Information Fig. S10A), and **HVA**, which demonstrated the most potent protective effect

expression of HDAC2 and ANP and increased the H3K9ac/H3 ratio in myocardial tissue. (L) Statistical analysis of the expression levels of HDAC2. (M) Statistical analysis of the acetylation level of histone 3. Protein expression was normalized to GAPDH, and the ratio to the sham group was calculated, $n = 3$. (N) Mechanistic illustration of how HVA and 2OH−VA alleviate cardiac hypertrophy. The dosage concentrations of HVA and 2OH−VA in I−M were 30 mg/kg. The data are presented as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ *vs.* Model; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#\#}P < 0.0001$ *vs.* Sham.

(Fig. S10B), were chosen as representative active metabolites for further investigation. It is noteworthy that, following administration of **HL0877i** to mice, we observed elevated concentrations of **HVA** and **2OH−VA** in both plasma and cardiac tissue, with **HVA** levels notably higher than those of **2OH−VA** (Supporting Information Fig. S11). **HVA** and **2OH−VA** have displayed promising protective effects against OGD-induced damage and ISO-induced cardiac hypertrophy (Supporting Information Fig. S12). In contrast to HF mice, the oral administration of 30 mg/kg of **HVA** or **2OH−VA** (Fig. 1H) significantly enhanced cardiac function, reduced inflammatory cytokines and BNP levels in serum, and mitigated cardiac damage and fibrosis (Supporting Information Fig. S13A−S13E). WGA staining revealed a significant reduction in cardiac hypertrophy (Fig. 1I and J). Moreover, both **HVA** and **2OH−VA** enhanced the grip strength and endurance capacity of the mice (Fig. S13F and S13G), indicating the potential to improve the prognosis of patients with HF.

4. HVA and 2OH−VA suppress the HDAC2−KLF4/INPP5F−AKT−GSK3β signaling pathway to ameliorate pathological hypertrophy

The metabolites significantly inhibit hypertrophy, a key process in HF pathogenesis, so we further investigated potential signaling pathways to elucidate how these metabolites alleviate hypertrophy. Given that veratric acid, a homolog of **HVA** and **2OH−VA**, has cardiovascular protective effects and is known to modulate the expression of histone deacetylases (HDACs)[4]. Initially, our findings indicated that the expression of HDAC2 was elevated in cardiomyocytes following OGD injury (Supporting Information Fig. S14A). This upregulation was significantly reduced by treatment with either **HVA** or **2OH−VA**, while the levels of HDAC1 and HDAC3 remained unchanged (Fig. S14B and S14C). The acetylation of histone H3K9, a substrate for HDACs, was upregulated by either **HVA** or **2OH−VA** (Fig. S14D). Similar results were observed in mouse myocardial tissues, where **HVA** and **2OH−VA** increased H3K9 acetylation levels by reducing HDAC2 expression (Fig. 1K−M). Then, we detected the expression of Krüppel-like factor 4 (KLF4) and inositol polyphosphate-5-phosphatase f (INPP5F), which inhibit the transcription and translation of atrial natriuretic peptide (ANP), resulting in anti-hypertrophic effects[5]. As depicted in Supporting Information Fig. S15A, the expression of both KLF4 and INPP5F was dramatically downregulated in the hypertrophic myocardium of HF-mice, while ANP expression was upregulated (Fig. 1K). **HVA** and **2OH−VA** could restore the physiological expression levels of these effector proteins. Moreover, INPP5F has been demonstrated to counteract hypertrophy, which leads to the activation of glycogen synthase kinase 3β (GSK3β) through the inactivation of protein kinase B (AKT). As illustrated in Fig. S15B, **HVA** and **2OH−VA** normalized elevated AKT and GSK3β phosphorylation levels in cardiac tissue. Collectively, these findings indicated that **HVA** and **2OH−VA** modulated the HDAC2−KLF4/INPP5F−AKT−GSK3β signaling pathway (Fig. 1N). Additionally, our data demonstrated that mice treated with **HL0877i** and subjected to FMT also regulate this pathway (Supporting Information Fig. S16), thereby contributing to anti-hypertrophy.

Collectively, our study first reported the therapeutic potential of the Chinese herb *Veronica anagallis-aquatica L.* in treating HF, and the iridoid glycosides (**HL0877i**) were the main active ingredients contributing to the anti-HF efficacy. Furthermore, **HL0877i** can reshape the gut microbiota and elevate the concentrations of the metabolites **HVA** and **2OH−VA**, thereby mitigating cardiac hypertrophy by HDAC2−KLF4/INPP5F−AKT−GSK3β signaling pathway. These findings imply that modulating the gut microbiome to ameliorate cardiac hypertrophy offers innovative strategies for HF treatment, with **HL0877i** emerging as a promising candidate in gut microbe-dependent therapeutic approaches.

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Author contributions

Manjiong Wang and Xiaobo Guo performed the research, analyzed the data, and wrote the main manuscript. Conglong Xia, Jian Li and Yixiang Xu designed the research study. Hanfang Liu, Xiao Li. and Yue Yao contributed to the efficacy evaluation *in vivo*. Conglong Xia, Qing Fu and Yu Jin contributed to the HPLC analysis, isolation and identification of components. Shuaishuai Ni, and Xiaokang Li contributed to the experimental design and drafting and critical revision of the manuscript. Chaojiang Xiao and Bei Jiang contributed to collection and extraction of Chinese herbs. The authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2025.04.005.

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