Heshouwu is a traditional non-toxic Chinese medicine commonly used in the clinical setting; however, clinical cases of Heshouwu-induced hepatotoxicity have been frequently reported [1]. Pharmacoepidemiological studies have found that Heshouwu-induced liver injury occurs only in a small fraction of individuals taking the drug; these patients presented with the typical characteristics of idiosyncratic drug-induced liver injury (IDILI). Human IDILI can be reproduced in animals by drug co-exposure to appropriate doses of lipopolysaccharide (LPS), which evokes modest inflammatory stress [2]. Many drugs that cause human IDILI, including ranitidine [3] and trovafloxacin [4], can also cause liver injury with a non-hepatotoxic dose in the LPS model.

The primary form of stilbene in Heshouwu is trans-stilbene glycoside (trans-SG), which is converted into cis-stilbene glycoside (cis-SG) by ultraviolet or natural light irradiation in Heshouwu. The content of cis-SG in Heshouwu is very low, but this content is significantly increased in most of the samples from Heshouwu-induced liver injury patients. This result revealed that cis-SG was positively correlated with Heshouwu-induced liver injury. We used a target constituent knock-out/knock-in strategy to track and subsequently demonstrate that cis-SG is the major perpetrator responsible for Heshouwu-induced idiosyncratic liver injury in rats [5]. Fourfold clinical doses of Heshouwu induced liver injury in the LPS model [6]; however, the cis-SG dose that caused liver damage in the LPS model was 50 mg/kg, which is the minimum equivalent to eight times the Heshouwu clinical dose. This suggests that cis-SG is not the only component in Heshouwu that can induce liver injury; rather, other constituents in Heshouwu may synergistically aggravate cis-SG-induced liver injury. According to previous research, emodin and trans-SG may represent other constituents in Heshouwu that can induce liver injury. Many studies have reported that emodin can inhibit LPS-induced inflammation within a certain dose range [7,8]; therefore, emodin may not have the effect of immunologic hepatotoxicity to enhance cis-SG-induced liver injury. Trans-SG, the major bioactive constituent of Heshouwu, has a variety of pharmacological effects, such as regulating immunity and inhibiting oxidative stress, and has no hepatotoxic effects in vivo, unlike the same dose of cis-SG, which results in liver injury in the LPS model. The content of trans-SG can be ten times higher than that of cis-SG in clinical samples of Heshouwu and has the potential to induce liver injury; therefore, whether the geometric ratio doses of trans-SG in Heshouwu will augment cis-SG-induced liver injury in the LPS model remains to be evaluated.

The chemical structures of cis-SG and trans-SG are shown in Supplementary Fig. 1, online. According to the previous determination of trans-SG and cis-SG in Heshouwu in DILI patients, we assumed that the ratio of cis-SG was 0.5% and the trans-SG ratio was 5% in Heshouwu. Therefore, we evaluated liver injury induced by cis-SG combined with trans-SG in the LPS model using a dose of 50 mg/kg of cis-SG and doses of 250 or 500 mg/kg of trans-SG. As shown in Fig. 1a, b, the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in cis-SG- (50 mg/kg) and trans-SG-treated rats (250 or 500 mg/kg) showed less change than the levels in normal rats. Co-treatment with LPS and cis-SG resulted in significantly increased ALT and AST serum levels, while co-treatment with LPS and trans-SG (250 or 500 mg/kg) did not induce ALT and AST elevation. Interestingly, compared with the levels in LPS/cis-SG-treated rats, the ALT and AST serum levels were significantly increased in LPS/cis-SG/trans-SG-treated rats.
Fig. 1. (Color online) Detection of alanine transaminase (ALT) and aspartate aminotransferase (AST) in rat serum (a, b). Hepatocyte apoptosis in rat by TUNEL assay (c). Integral optical density values of p65 (d). The immunological stress-mediated tri-element injury hypothesis of idiosyncratic toxicity of traditional Chinese medicines (e) [10]. The mechanism of Heshouwu induced idiosyncratic liver injury (f). The results are given as mean ± SD (n = 10, * P < 0.05 vs LPS group, ** P < 0.01 vs LPS group. ^ P < 0.05 vs LPS/cis-SG group. P < 0.05 vs Control group).
Regarding the histopathological analysis shown in Supplementary Fig. 2, there was no obvious pathological change in animals treated with cis-SG or trans-SG alone or with trans-SG combined with LPS. Cis-SG combined with LPS showed an increase in macrophages in liver tissue and a widening of the hepatic sinus. LPS/cis-SG-treated rats and LPS/trans-SG-treated rats exhibited obvious pathological changes, including central vein inflammatory cell infiltration, widening of the hepatic sinus, and vascular degeneration. TUNEL staining (Fig. 1c, Supplementary Fig. 3) was used to evaluate hepatocyte apoptosis in LPS, cis-SG, trans-SG, LPS/cis-SG, LPS/trans-SG and LPS/cis-SG/trans-SG-treated rats. We detected few apoptotic cells in the livers of rats treated with LPS, cis-SG or trans-SG. Although cis-SG combined with LPS induced the apoptosis of hepatic cells, the effect of trans-SG on the apoptosis of hepatic cells was not significant in the LPS model. Compared to that in all other groups, the liver injury in the LPS/cis-SG/trans-SG-treated rats was more severe; in particular, far greater numbers of apoptotic cells were detected in the liver tissues of LPS/cis-SG/trans-SG-treated rats than in the tissues of LPS/cis-SG-treated rats. These results revealed that trans-SG further aggravated LPS/cis-SG-induced liver injury in rats.

Inflammatory cell infiltrates, including neutrophils and macrophages, may be either the cause or result of drug-induced liver injury and constitute one of the main features of this type of injury. Indeed, the mild inflammation that occurs during the course of drug therapy markedly decreases the threshold for drug hepatotoxicity. Over the past few years, many drugs that cause human IDU have also been shown to cause liver injury and inflammatory cell infiltration in animals co-treated with a nonhepatotoxic dose of LPS [9]. To further investigate the combined effects of trans-SG and cis-SG in the LPS model, we detected macrophage infiltrates by immunohistochemical staining with the macrophage-specific marker CD68 (Supplementary Fig. 4). The results indicated that, compared with LPS treatment, treatment with trans-SG alone did not cause macrophages to infiltrate into the liver tissue. Nevertheless, trans-SG significantly increased macrophage infiltration in LPS/cis-SG/trans-SG-treated rats compared with that in LPS/cis-SG-treated rats.

Based on the above data, trans-SG alone or LPS in combination with trans-SG did not result in persistent hepatocyte apoptosis and macrophage infiltration. These results indicated that trans-SG could not induce hepatocyte apoptosis nor change the proportion of immune cells in the liver tissue. To elucidate the mechanism underlying the synergistic effect of trans-SG and cis-SG in the LPS model, we detected the expression of several immune and inflammatory cytokines using enzyme-linked immunosorbent assays (ELISAs) (Supplementary Fig. 5) and RT-qPCR (Supplementary Fig. 6). Compared with LPS-treated rats, in LPS/trans-SG-treated rats, the protein and mRNA expression levels of IL-1β, IL-6, IFN-γ and TNF-α were significantly increased in a dose-dependent manner; however, this result was not observed in trans-SG-treated rats. Interestingly, the expression of these cytokines was significantly increased in LPS/cis-SG/trans-SG-treated rats compared to the expression levels in LPS/cis-SG-treated rats. Together, these results suggest that trans-SG may enhance the expression of immune inflammatory cytokines to aggravate LPS/cis-SG-induced liver injury.

NF-kB signalling plays a critical role in modulating the expression of immune inflammatory cytokines in the LPS model. The expression of p65 was detected by immunohistochemical staining (Fig. 1d, Supplementary Fig. 7). Compared with the expression in the control group, the expression of p65 was not significantly altered following treatment with cis-SG or trans-SG alone. However, consistent with previous results, cis-SG significantly increased the expression of p65 in the LPS model. In the present study, we found that the expression of p65 also increased in the livers of rats treated with LPS/trans-SG compared with that in the LPS model group. We also detected a significant increase in the expression of p65 in LPS/cis-SG/trans-SG-treated rats compared with that in the LPS/cis-SG-treated rats. These data confirm the promoting effect of trans-SG on the expression of p65 in LPS/trans-SG or LPS/cis-SG/trans-SG-treated rats.

In this research, we evaluated whether trans-SG aggravates cis-SG-induced liver injury in an LPS model with a dose of 50 mg/kg of cis-SG, and doses of 250 or 500 mg/kg of trans-SG respectively, which is an experiment based on the premise that Heshouwu contains 0.5% cis-SG and 2.5% or 5% trans-SG. The results are consistent with our expectations that trans-SG aggravates LPS/cis-SG-induced liver injury in a dose-dependent manner. Trans-SG alone or in combination with LPS did not cause liver injury in rats; however, we found that trans-SG significantly aggravated cis-SG-induced liver injury in an LPS model, as confirmed by liver function analysis, H&E staining, TUNEL assay and analysis of infiltrating inflammatory cells. Trans-SG alone slightly increase the cytokines levels, while trans-SG alone did not cause macrophages to infiltrate into the liver tissues, and the expression of p65 increased in the liver of rats treated with LPS/trans-SG compared with the LPS model group. Meanwhile, the expression of p65 significantly increased in LPS/cis-SG/trans-SG-treated rats compared with LPS/cis-SG-treated rats. These results indicate that trans-SG play a role mainly by activating immune cells, not infiltrating inflammatory cells. trans-SG did not promote liver damage. LPS/trans-SG significantly promoted the expression of immune inflammatory cytokines IL-1β, TNF-α, IL-6, IFN-γ and the expression of p65, indicating that trans-SG can improve LPS-mediated immune inflammatory activity in rats. We found that not only immune inflammatory activity but also apoptosis were significantly increased in LPS/cis-SG/trans-SG-treated rats compared with LPS/trans-SG-treated rats and LPS/cis-SG-treated rats. These results indicated that trans-SG aggravated LPS/cis-SG-induced liver injury by improving immune inflammatory activity.

Based on previous studies and the data presented here, we show that cis-SG is the major perpetrator responsible for Heshouwu-induced liver injury, while trans-SG is another cause of Heshouwu-induced liver injury. Therefore, we demonstrated the immunological stress-mediated tri-element injury hypothesis of idiosyncratic toxicity of Heshouwu that when the body is in an immune stress state, the hepatotoxicity thresholds for cis-SG are altered to induce liver injury, meanwhile, trans-SG enhances the immune response to further aggravate cis-SG-induced liver injury (Fig. 1e, f). The result not only comprehensively elaborates the immune synergy mechanism of Heshouwu-induced idiosyncratic liver injury but also provides a foundation for future research of similar traditional Chinese medicines.

Conflict of interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

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References


