从代谢角度探讨中药抗药源性肝损伤机制及相关效应物质

黄心慧[1]，周婷婷[1,2*]

(1. 海军军医大学药学院, 上海 200433;　2. 上海市药物 (中药) 代谢产物研究重点实验室, 上海 200433)

摘要: 药源性肝损伤是临床常见的药物不良反应之一, 且时至今日仍然是药物限制使用、研发终止和上市后警告撤市的主要原因。近年来, 中药中多种化学成分及代谢产物和二者通过代谢影响的内源性效应物质因具有良好的保肝活性备受关注, 但目前中药抗药源性肝损伤的机制复杂、效应物质尚不明确, 且其与代谢相关的研究仍比较薄弱。因此本文从代谢的角度对药源性肝损伤的机制以及其中药治疗的机制进行综述, 并首次创新性地将中药效应物质分为外源性 (中药活性成分及代谢物) 和内源性 (肠道益生菌、内源性代谢产物) 两大类, 为减少药源性肝损伤发生, 探索并开发有效的中药抗药源性肝损伤效应物质, 并进一步研制具有肝保护作用的临床药物提供参考。

关键词: 药源性肝损伤; 中药治疗; 作用机制; 效应物质; 代谢

中图分类号: R917　　**文献标识码**: A　　**文章编号**: 0513-4870(2023)03-0646-13

Investigation on the mechanism of anti-drug-induced liver injury and related effector substances of traditional Chinese medicine from the perspective of metabolism

HUANG Xin-hui[1], ZHOU Ting-ting[1,2*]

(*1. School of Pharmacy, Navy Military Medical University, Shanghai 200433, China;　2. Shanghai Key Laboratory for Pharmaceutical (Chinese Materia Medica) Metabolite Research, Shanghai 200433, China*)

Abstract: Drug-induced liver injury (DILI) is one of the common clinical adverse drug reactions and remains a major cause of drug restriction, development termination and withdrawal from the pharmaceutical market today. In recent years, a variety of chemical components and metabolites of traditional Chinese medicine (TCM), as well as the endogenous effector substances influenced by metabolism of both, have attracted much attention for their significant hepatoprotective activities. However, the mechanism of TCM against DILI is complex, the related effector substances are still unclear, and its metabolism-related studies are still relatively weak. Therefore, this review summarized the mechanisms of DILI and its treatment by TCM from the perspective of metabolism, and for the first time, innovatively classified the Chinese medicine effector substances into two categories: exogenous (active components and metabolites of TCM) and endogenous (intestinal probiotics and endogenous metabolites), in order to reduce the occurrence of DILI, explore and develop effective anti-drug-induced liver injury effector substances of TCM, and further develop clinical drugs with hepatoprotective effects.

Key words: drug-induced liver injury; treatment by traditional Chinese medicine; mechanism; effector substance; metabolism

肝脏是参与内源性、外源性物质解毒和代谢的主要器官。药源性肝损伤 (drug-induced liver injury, DILI) 指药物及其代谢产物诱发, 或由于机体对药物及其代谢产物耐受性降低或敏感性过高导致的肝损伤, 可由化学药物、天然药物、中草药、生物制剂、保健品等及其代谢产物引起。药源性肝损伤患者的临床症状十分广

收稿日期: 2022-08-18;　修回日期: 2022-12-28.

基金项目: 国家自然科学基金资助项目 (81973457).

*通讯作者 Tel: 86-21-81871262, E-mail: tingting_zoo@163.com

DOI: 10.16438/j.0513-4870.2022-0999

泛, 常表现为肝酶异常升高、肝炎、肝细胞坏死、胆汁淤积、脂肪肝和肝硬化等, 且难与其他肝脏疾病区分。时至今日, 药源性肝损伤仍然是药物限制使用、研发终止和上市后警告撤市的主要原因[1]。药源性肝损伤发生的机制较为复杂, 但近期研究发现其与代谢密切相关。药物及其代谢产物除直接改变药物代谢酶活性以影响自身或联用药物的代谢特征外, 还可以通过干扰内源性物质代谢引发肝损伤。

近年来, 因其在治疗和预防疾病中的显著疗效, 中草药及其提取物获得世界上广泛认可。大量研究[2]表明, 中药中多种化学成分如多糖、黄酮、萜类等都具有良好的肝保护活性。中药可以原形或代谢物形式直接作用于靶点发挥肝保护作用; 同时, 也可以通过调节肠道菌群结构丰度进而改变肠道代谢特征, 调节肠道菌群释放的次生代谢产物等内源性物质发挥作用。

由此可见, 药源性肝损伤的机制及中药发挥肝保护作用机制都与代谢密切相关, 因此本文从代谢的全新视角综述药源性肝损伤产生机制以及中药治疗的潜在机制, 并基于此, 首次创新性地将中药效应物质分为外源性 (中药活性成分及代谢物) 和内源性 (肠道益生菌、内源性代谢产物) 两大类, 以期为减少药源性肝损伤、探索并开发有效的中药抗药源性肝损伤效应物质, 并进一步研制具有肝保护作用的临床药物提供参考。

1　药源性肝损伤及其中药治疗机制

在药物体内代谢过程中, 药物及其代谢产物可能影响药物代谢酶 (如I相代谢酶、肠道菌群来源酶等) 的水平或活性, 进而影响药物自身或联用药物的代谢特征, 易引发肝损伤; 除此之外, 机体内源性物质的代谢也受到药物代谢过程的影响, 若内源性物质稳态被破坏, 肝损伤风险随之增加。药源性肝损伤形成机制目前还未完全阐明, 但根据目前大量的体内外实验研究, 药源性肝损伤的机制 (图1) 可能与药物代谢酶表达异常、氧化应激损伤、炎症反应、肠道菌群紊乱有关。

中药、中药提取物及中药单体成分进入体内后, 不仅可影响药物代谢酶的水平和活性, 进而影响肝损伤药物的代谢, 还可能调节机体内源性物质代谢, 使内源性物质和代谢过程回调到平衡状态, 从而发挥治疗作用。其保护作用主要通过调控药物代谢酶、抗氧化应激、抗炎、调控肠道菌群等实现。表1[3-37]总结了抗药源性肝损伤的中药单体化合物或提取物, 并简单介绍了其发挥肝保护作用的机制。

1.1　调控药物代谢酶

1.1.1　药物代谢酶表达异常引发的药源性肝损伤　药物进入体循环后, 首先会在肝脏中药物代谢酶作用下发生生物转化。其中I相代谢反应是药物代谢的限速步骤, 并参与药物的解毒与致毒。细胞色素P450酶 (cytochrome P450, CYP450s) 由CYP基因调控, 是I相代谢酶的重要组成部分[38], 部分药物可以通过抑制或诱导CYP450酶的活性或表达水平, 减缓或加快药物代谢速率, 引起药物或代谢物蓄积, 并进一步导致肝毒性。

雷公藤甲素 (triptolide, TP) 可剂量依赖性地阻断CYP3A4的转录激活。阿托伐他汀为一种常用降血脂药物, 通过CYP3A4代谢消除, 其引发肝损伤与用药剂量及体内蓄积相关[39]。当二者联合用药时, 阿托伐他汀的代谢消除受到抑制, 其体内暴露量增加, 肝损伤风险加大[40]。

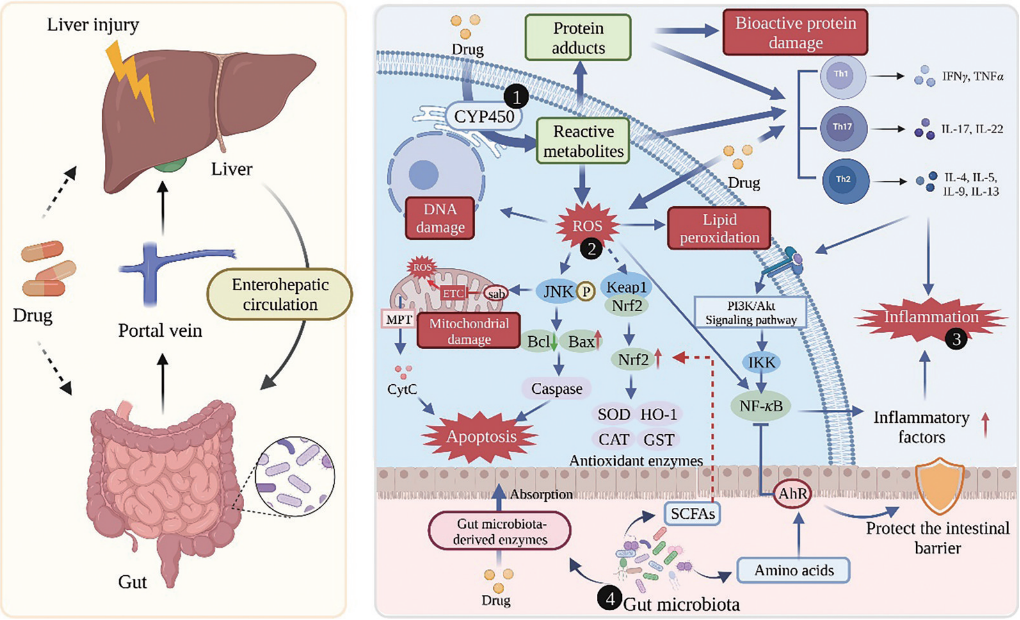

Figure 1　Mechanism of drug-induced liver injury generation

Table 1 Summary of the effects of active components of TCM against drug-induced liver injury

Name of herb or compound	Model	Liver injury model	Molecular mechanism	Ref.
Aqueous extract of Radix Bupleuri	ICR mice, Wistar rats	Acetaminophen (APAP)	Inhibited the increase of activity and translation of cytochrome P450 enzymes	[3]
Berberine	Wistar rats	Methotrexate	Activated Nrf2/HO-1 pathway and PPARγ, and suppressed oxidative stress and apoptosis	[4]
Ferulic acid	Wistar rats	Methotrexate	Activated Nrf2/HO-1 signaling and PPARγ, and attenuated oxidative stress, inflammation, and cell death	[5]
50% ethanol extract of Gardeniae Fructus, 50% ethanol extract of Zhizichi decoction	Sprague-Dawley rats, HepG2 cells	Genipin	Regulated the microbiota, promoted butyrate production, and activated antioxidant response	[6]
Allicin	Sprague-Dawley rats	Cyclophosphamide	Activated the Nrf2 signaling pathway and downstreamed antioxidant genes that attenuate oxidative insult and inflammatory and apoptotic responses	[7]
Ethanol extract of licorice (*Glycyrrhiza uralensis*)	HepG2 cells, ICR mice	Triptolide	Activated the Nrf2 signaling pathway and increased the level of Nrf2 and its downstream genes	[8]
Liquorice aqueous extract, 18β-Glycyrrhetinic acid	Sprague-Dawley rats	Retrorsine	Inhibited activities of CYPs, especially CYP3A1, the major CYP isoform responsible for the metabolic activation of RTS in rats	[9]
Glycyrrhetinic acid	BALB/c mice	Acetaminophen	Down-regulated CYP2E1 expression, refreshed the content of GSH, inhibited APAP-induced pro-inflammatory cytokines production	[10]
Cordyceps sinensis polysaccharides, *Ganoderma atrum* polysaccharides	BALB/c mice	Cyclophosphamide	Enhanced modulations on cellular oxidant/antioxidant imbalance, mitochondrial apoptotic pathway and pro-inflammatory factors	[11]
Chrysin	Wistar rats	Cyclophosphamide	Inhibited oxidative stress, apoptosis, inflammation, and autophagy	[12]
Berberine	C57BL/6 mice	Acetaminophen	Inhibited oxidative stress, hepatocyte necrosis and inflammatory response	[13]
Laminaria japonica fucoidan	ICR mice	Cyclophosphamide	Up-regulating the Nrf2/HO-1 pathway and inhibiting the TLR4/NF-κB pathway	[14]
Galangin	Wistar rats	Cyclophosphamide	Activated Nrf2/HO-1 signaling and attenuated oxidative damage, inflammation, and cell death	[15]
Gallic acid	Wistar rats	Diclofenac	Reduced cellular ROS generation, restored enzymatic and non-enzymatic antioxidants, as well as improved liver function enzymes	[16]
Ginseng saponins	Sprague-Dawley rats	Cyclophosphamide	Upregulated CYP2B6, CYP2C9, and CYP3A4 protein expression in rat livers, lessened oxidative stress, inhibited inflammatory factors	[17]
Naringin, hesperidin	Wistar rats	Diclofenac	Antioxidant, anti-inflammatory, and antiapoptotic actions	[18]
Glabridin	Swiss mice	Methotrexate	Attenuated oxidative stress, inflammation, and apoptosis	[19]
Naringin	Wistar rats	Cyclophosphamide	Increased antioxidant enzyme activities, and regulated inflammation, apoptosis, autophagy, and oxidative DNA damage in hepatic tissues	[20]
Silymarin	Wistar rats	Diclofenac	Reduced oxidative stress	[21]
Silymarin	Wistar rats	Triptolide	Improved antioxidant and anti-inflammatory status, as well as prevented hepatocyte apoptosis	[22]
Saikosaponin d	C57BL/6 mice	Acetaminophen	Down-regulated NF-κB-mediated inflammatory signaling	[23]
Quercetin	Wistar rats	Cyclophosphamide	Activation of Nrf2/HO-1 signaling pathway with subsequent suppression of oxidative stress and inflammation	[24]
Saffron stigma alcoholic extract	Wistar rats	Vincristine sulfate	Reduced antioxidant depletion and lipid peroxidation, presumably due to its antioxidative properties	[25]
Processed *Aconitum carmichaelii*	HepaRG cells	Acetaminophen	Inhibited CYP2E1 activity, GSH depletion, and mitochondrial dysfunction	[26]
Dendrobium officinale polysaccharides	ICR mice	Acetaminophen	Suppressed the oxidative stress and activated the Keap1-Nrf2 signaling pathway	[27]
Baicalein, baicalin	L-02 cells, C57BL/6 mice	Acetaminophen	Suppressed the oxidative stress and activated Nrf2 *via* blocking the binding of Nrf2 with Keap1 and inducing Nrf2 phosphorylation	[28]
Salvianolic acid B	C57BL/6 mice	Senecionine	Suppressed the oxidative stress	[29]
Hyperoside	Kunming mice	Acetaminophen	Increase activities and mRNA expressions of uridine diphoshate glucuronosyltransferases and sulfotransferases, as well as to inhibit CYP2E1 activities, and thereby suppressed toxic intermediate formation and promoted APAP hepatic detoxification	[30]
Dendrobine	ICR mice	Isoniazid and rifampicin	Regulated oxidative stress status in the liver by the regulation of CYP1A2	[31]
Poria Cocos polysaccharides	Kunming mice	Acetaminophen	Suppressing inflammatory response and apoptosis in liver cells	[32]

Continued

Name of herb or compound	Model	Liver injury model	Molecular mechanism	Ref.
Schisandra chinensis acidic polysaccharide	ICR mice	Acetaminophen	Inhibited oxidative stress, inflammation, and cellular apoptosis	[33]
Schisandra chinensis polysaccharide	ICR mice	Acetaminophen	Improved antioxidant capacity and inhibited apoptosis, possibly related to inhibition of JNK signaling pathway activation	[34]
Platycodon grandiflorum saponins	ICR mice	Acetaminophen	Activated AMPK/PI3K/Akt signaling pathway and reduced NF-κB signaling pathway, inhibited oxidative stress, inflammation, and cellular apoptosis	[35]
Sophorae tonkinensis Radix Polysaccharides	ICR mice	Acetaminophen	Suppressed the oxidative stress	[36]
Wuzhi Tablet	C57BL/6 mice	Acetaminophen	Inhibited the activity of CYP2E1 and CYP3A11, which related to APAP metabolic activation	[37]

1.1.2 中药通过调控药物代谢酶减轻药源性肝损伤

肝脏中药物代谢酶与药物的解毒及毒性产生密切相关, 药物代谢酶表达水平与活性直接影响药物的代谢速率和途径, 因此通过调控药物代谢酶是中药发挥肝保护作用的重要机制。

CYP2E1 和 CYP3A 将对乙酰氨基酚 (acetaminophen, APAP) 代谢成肝毒性物质 *N*-乙酰基对苯醌亚胺 (*N*-acetyl-*p*-benzoquinone, NAPQI), 当柴胡水提物与 APAP 联合使用时, CYP2E1 和 CYP3A 蛋白表达受到显著抑制的同时, 其基因表达无显著变化, 表明柴胡水提物可通过抑制 CYP450 活性来抑制 APAP 代谢发挥肝保护作用[3]。制草乌中主要活性成分为乌头类生物碱, 绝大部分由 CYP1A、CYP2C、CYP3A 介导代谢。草乌能抑制 CYP1A 及 CYP3A 的活性, 下调体内 CYP1A2、CYP2C11、CYP3A1 mRNA 水平, 减缓代谢, 在体内蓄积产生毒性。而诃子、甘草与其配伍后可上调 CYP1A2、CYP2C11、CYP3A1 mRNA 的水平, 加快代谢, 降低体内暴露量, 减轻肝损伤[41]。

1.2 抗氧化应激

1.2.1 氧化应激引发的药源性肝损伤 氧化还原过程与细胞能量代谢密切相关, 是机体生命活动不可或缺的部分, 支持许多重要的生理过程。正常生理状况下, 活性氧自由基 (reactive oxygen species, ROS), 如氧自由基、羟基自由基、过氧自由基等由机体代谢产生, 同时过量的 ROS 又被谷胱甘肽 (glutathione, GSH)、过氧化氢酶 (catalase, CAT)、超氧化物歧化酶 (superoxide dismutase, SOD)、谷胱甘肽过氧化物酶 (glutathione peroxidase, GPx) 等组成的抗氧化系统清除, 以保证活性氧自由基在体内保持相对稳态。氧化应激状态下, 机体 ROS 水平提高, 抗氧化系统清除能力下降, 蛋白质、DNA、脂质等生物大分子可能受到不可逆损伤, 最终导致细胞死亡[1]。部分药物通过破坏肝脏 ROS 代谢平衡, 使肝脏长期处于氧化应激状态下, 最终导致肝损伤。

氧化应激状态下, ROS 攻击细胞膜, 从脂质中去除电子, 产生活性中间体, 激活连锁增殖反应, 形成一系列脂质自由基及其降解产物丙二醛 (malondialdehyde, MDA) 的过程被称为脂质过氧化。脂质过氧化过程不仅直接损害膜磷脂, 其过氧化链式反应产物也具有较高生物活性, 可以破坏 DNA、蛋白质和酶的生物活性, 也可作为细胞死亡信号诱导程序性细胞死亡[42]。阿霉素作为化疗药物广泛用于各种癌症的治疗, 其在急性、亚急性剂量下使用均可能引发肝毒性[43]。阿霉素在体内可通过 NADPH 参与的酶途径, 或三价铁离子参与的非酶途径代谢产生大量活性氧, 攻击膜中多不饱和脂肪酸、DNA、蛋白质, 并进一步引起一系列的凋亡事件[44]。

大量研究表明, 线粒体已成为诱发药源性肝损伤的重要靶点。药物及其代谢产物可通过与线粒体蛋白结合或消耗线粒体 DNA[45]等方式抑制电子传递链, 线粒体能量代谢紊乱, 导致 ROS 累积, 线粒体膜通透性转运孔异常开放, 导致线粒体膜受损, 促凋亡因子释放, 最终引发肝细胞凋亡。以对乙酰氨基酚所致肝毒性为例, 其经 CYP2E1 和 CYP3A 酶代谢后的活性代谢产物 NAPQI 与包括线粒体蛋白在内的细胞蛋白质巯基共价结合, 形成药物-蛋白加合物, 抑制电子传递链, 导致线粒体内 ROS 水平升高, 最终导致 DNA 损伤, 肝细胞坏死[46]。类似地, 丙戊酸中断线粒体呼吸链的稳态, 触发 JNK 信号通路, 上调 ROS 水平, 线粒体膜通透性改变, 最终引发肝细胞死亡[47]。

1.2.2 中药通过抗氧化应激减轻药源性肝损伤 目前中药抗肝损伤的机制中, 关于抗氧化应激的研究较为集中, 主要机制是中药效应物质通过影响代谢, 提高细胞抗氧化能力, 减轻 ROS 对 DNA、膜蛋白质等的损伤, 保护线粒体、细胞膜及细胞器膜, 减轻肝损伤[48]。

研究[49]表明, 许多中药抗药源性肝损伤与色氨酸代谢途径密切相关, 而 5-羟色胺、褪黑素、犬尿氨酸等色氨酸途径相关代谢物均有良好的抗氧化应激活性。

Gao 等[50]研究二氢杨梅素抗大黄素致肝损伤的机制，发现口服二氢杨梅素可通过改善肝代谢以减轻肝损伤，其中色氨酸代谢是受影响最显著的途径之一。在大黄素致肝损伤模型中，犬尿氨酸被显著下调，而给予二氢杨梅素后回调至正常水平。作为参与色氨酸代谢途径的重要代谢物之一，犬尿氨酸体内水平降低可能引发肝功能障碍。犬尿氨酸可被犬尿酸酶进一步代谢为 3-羟基-2-氨基苯甲酸 (3-hydroxyanthranilic acid, HA)，HA 可作用于 Keap1-Nrf2 通路，改变 Keap1 的构象，诱导 Nrf2 和 Keap1 解离，激活 Nrf2 进入细胞核，诱导下游编码抗氧化和解毒酶的基因转录表达，包括 SOD、CAT、NQO1、HO-1、GST 等，从而提高细胞抗氧化能力，清除过量 ROS，恢复细胞氧化还原稳态，减轻 ROS 累积对肝细胞的损伤[49]。

1.3 抗炎

1.3.1 炎症反应引发的药源性肝损伤 肝脏所代谢的外源物质易作为抗原，诱发肝脏炎症反应，促进细胞因子大量释放。药物或活性代谢物除了直接与人类白细胞抗原非共价结合外，某些药物代谢物可以作为半抗原与内源性蛋白共价结合形成新抗原诱发免疫应答，促进细胞因子释放，最终导致免疫特异性肝损伤。异烟肼的活性代谢物与肝蛋白结合形成加合物可能与免疫反应及异烟肼诱发的肝损伤相关。有研究证实在小鼠肝脏与人肝微粒体中存在异烟肼衍生的蛋白共价加合物[51]。此外，也有异烟肼与 CYP2E1、CYP3A4 和 CYP2C9 形成共价加合物的相关报道，19 例异烟肼诱导肝衰竭患者血清中可检测到抗异烟肼抗体、抗 CYP2E1 抗体、抗 CYP3A4 抗体、抗 CYP2C9 抗体以及抗异烟肼修饰的 CYP2E1 抗体，而服用异烟肼但无明显肝损伤患者中则未检测到[52]。这些数据为异烟肼诱导免疫反应导致肝损伤提供了强有力的支撑。

代谢和免疫系统是相互依赖且高度相关的，代谢稳态改变可能导致免疫功能障碍，导致肝脏对药物毒性更加敏感，从而增加肝毒性的风险。Tu 等[53]研究发现，双氯芬酸致肝损伤大鼠体内免疫异常主要与鞘脂、酪氨酸和胆汁分泌代谢等途径的上调有关。其中，氨基酸代谢在免疫和炎症反应中起着至关重要的作用，炎症应激状态下，氨基酸代谢与免疫细胞增殖和免疫反应蛋白质的合成密切相关；一些鞘脂类神经酰胺，如鞘氨醇-1-磷酸和神经酰胺-1-磷酸，广泛参与免疫信号反应和转导；胆汁酸也可在 NLRP3 炎症小体的起始和激活阶段协同参与信号通路的激活，分泌促炎细胞因子 IL-1β，诱导炎症反应。

此外，氧化还原稳态失衡时，过量的 ROS 可以直接激活枯否细胞中的转录因子核因子-κB (nuclear factor kappa B, NF-κB)，促进炎症因子的转录表达，加剧肝损伤。相反，炎症反应则会通过影响 CYP450 酶的表达和活性来改变药物体内代谢，进而影响药物的体内毒性。促炎因子的释放降低 CYP450 酶的 mRNA 和蛋白表达水平及活性；NF-κB 可以直接或间接调控 CYP450 的表达与活性。这可能导致药物在体内清除障碍或药物蓄积，增加肝损伤风险[48]。

1.3.2 中药通过抗炎减轻药源性肝损伤 多种肝毒性药物与肝脏 NF-κB 表达及 TNF-α 等炎症因子水平上升有关。许多研究表明，舒林酸、曲伐沙星、雷尼替丁、胺碘酮等处理后，血浆中 TNF-α 浓度升高，TNF-α 清除率降低，其诱导的炎症反应加重，细胞死亡信号增强，肝损伤加重；TNF-α 抑制剂处理后肝损伤减轻，表明炎症应激条件下，血浆 TNF-α 浓度升高可能与这些药物诱导肝损伤有关[48]。中药可以通过激活过氧化物酶体增殖物激活受体 (peroxisome proliferator activated receptor gamma, PPARγ) 抑制 NF-κB 及其调控的炎症因子的释放，降低血浆中 TNF-α 浓度，从而减轻炎症[4]。Mahmoud 等[5]发现，阿魏酸除了可以抗氧化应激外，还可通过减轻炎症起到肝保护作用，减轻甲氨蝶呤引发肝损伤。与单独给予甲氨蝶呤的组别相比，预先给药阿魏酸的组别血清中 NF-κB、TNF-α 和 IL-1β 水平下降，而 PPARγ mRNA 和蛋白的表达增加，表明阿魏酸可通过激活 PPARγ，抑制 NF-κB、TNF-α 和 IL-1β 的释放，减轻炎症。

同时，已有线索表明，中药可以通过恢复胆汁分泌等代谢过程的稳态以减轻药源性肝损伤。人参给药显著逆转了环磷酰胺诱导肝损伤大鼠体内炎症介质的水平，表现出优越的抗炎及肝保护作用，其机制可能与调节胆汁酸代谢紊乱有关[54]；黄芪多糖调节初级胆汁酸的生物合成和甘油磷脂代谢，显著下调相关促炎物质，从而减少金丝桃苷引起的肝损伤[55]。

1.4 调控肠道菌群

1.4.1 肠道菌群紊乱引发的药源性肝损伤 人体内大约有 100 万亿个微生物，其中约 80% 分布于胃肠道中，其编码的酶广泛参与药物代谢。目前许多肝脏疾病 (如酒精性肝病、非酒精性脂肪肝病等) 与肠道菌群改变的联系已被证明，肠道菌群稳态对于预防和治疗药源性肝损伤至关重要[56]。

菌群结构紊乱与药源性肝损伤密切相关。Huang 等[57]研究表明，预先进行抗生素治疗消耗肠道菌群的 C57BL/6J 小鼠肝损伤显著加重，并在给予相对安全剂量的 TP (0.5 mg·kg^{-1}) 后死亡，且这一现象可由肠道菌群移植逆转。Schneider 等[58]为验证肠道菌群对药源性肝损伤作用，将肠道菌群紊乱小鼠 *Nlrp6*$^{-/-}$小鼠肠道

微生物群经粪菌移植转移至野生型小鼠中。粪菌移植后的野生型小鼠肠道菌群发生紊乱，菌群结构显著改变，且更接近于*Nlrp6*$^{-/-}$小鼠肠道菌群结构，经APAP诱导后肝损伤加重，表明肠道菌群紊乱可能加重药源性肝损伤。

肠道菌群紊乱影响酶的产生或活性，从而导致药物蓄积，引发肝毒性。甲氨蝶呤作为一种化疗药物，长期使用会引起肝毒性等一系列不良反应。甲氨蝶呤吸收后经肝脏中代谢为7-羟基甲氨蝶呤，并最终抑制嘌呤和嘧啶的合成以及下游DNA的合成，产生毒副作用。而肠道菌群可以参与后续解毒过程，7-羟基甲氨蝶呤经胆汁排泄再次进入胃肠道，在细菌羧肽酶G2 (carboxypeptidase glutamate 2, CPDG2) 作用下代谢为2,4-二氨基-*N*-10-甲氨蝶呤酸 (2,4-diamino-*N*-10-methylpteroic acid, DAMPA) 及其衍生物，从而发挥解毒作用。而Letertre等[59]发现长期暴露于甲氨蝶呤时大鼠肠道微生物群的群落和功能发生变化，甲氨蝶呤和DAMPA的排泄时间延长，且高剂量组大鼠DAMPA排泄量更低。这表明暴露于高剂量甲氨蝶呤后，微生物群落结构的变化可能会降低CPDG2的产生或活性，影响大鼠对甲氨蝶呤的代谢能力。

1.4.2　中药通过调控肠道菌群减轻药源性肝损伤　肠道菌群失调会引起肠道代谢酶及菌群代谢产物水平改变，并进一步影响药物代谢途径，引发肝损伤。而中药口服经过胃肠道，首先与肠道菌群接触并相互作用，因此可以通过调控肠道菌群结构，回调酶及代谢物水平，影响药物代谢途径，起到减毒增效作用。因此靶向肠道菌群已经逐渐成为中药抗药源性肝损伤治疗的新思路。

京尼平苷是栀子的最主要化学成分，其经肠道中β-葡萄糖苷酶代谢的产物京尼平通过多种机制产生肝毒性[60]。与栀子可能引发肝损伤不同，栀子豉汤作为《伤寒论》中的经典古方，未见其应用的肝毒性报道。研究[6]表明淡豆豉可调节肠道微生物群结构，上调益生菌*Lactobacillus*、*Akkermansia*、*Prevotella*等水平，下调机会致病菌*Enterococcus*等的水平，同时影响宿主代谢，加快京尼平苷在β-葡萄糖苷酶作用下转化为京尼平，并进一步加快其在II相代谢酶作用下转化为京尼平硫酸盐或京尼平葡醛酸结合物的速度，京尼平的体内暴露量降低，肝毒性减轻[61]。此外，Luo等[6]研究表明京尼平引发肝损伤与肝脏过氧化应激有关，而配伍淡豆豉可以调节肠道菌群结构，上调肠道菌群代谢物丁酸的水平，从而增强Nrf2在肝脏中表达，发挥抗氧化应激和抗炎作用，预防京尼平致肝损伤[6]。

2　中药抗药源性肝损伤效应物质

发挥抗药源性肝损伤作用的中药效应物质包括外源性和内源性两部分。中药活性成分及代谢产物即指外源性效应物质，其发挥肝保护作用的机制主要包括两类，一类是以原形或以活性代谢产物形式直接作用于靶点发挥作用；另一类是通过影响抗肝损伤的内源性效应物质来发挥作用，如调节代谢过程以改变内源性代谢产物水平；或调节肠道菌群结构丰度及肠道菌群来源代谢酶的水平，进而影响菌群次生代谢产物的产生，最终改变内源代谢产物水平。肠道益生菌及内源代谢产物即作为内源性效应物质发挥抗肝损伤作用，若单独给予相关的内源代谢产物可直接发挥肝保护作用；若给予相关的肠道益生菌可代谢产生内源代谢产物，同样发挥疗效。

2.1　外源性效应物质

中药活性成分及其代谢产物属于外源性效应物质，对中药活性成分及其代谢产物的研究是阐释中药作用机制的重要基础，也是目前研究的热点内容。目前针对外源性效应物质的研究主要集中两方面，一是治疗药物预先给药后用肝毒性药物诱发肝损伤，用于评价药物的预防解毒能力；二是治疗药物与肝损伤药物同时给药或预先给予肝毒性药物诱发肝损伤后给予药物治疗，以评价其肝损伤治疗作用。

随着技术和理论的不断革新，以中医整体、系统的理念为指导来寻找效应物质及研究作用机制成为近年中药效应物质研究的主要内容，“化学物质组学表征中药及复方-基因组学、蛋白质组学、代谢组学表征机体-生物标志物表征疾病症状”的研究模式逐渐形成[62]。作为继基因组学、转录组学和蛋白质组学之后出现的一种技术，代谢组学主要研究机体在生理和病理状态下或接受外源性药物治疗后，体内内源性物质变化规律。此外，作为计算机虚拟筛选的重要部分，网络药理学将基因组学、转录组学、蛋白质组学、代谢组学和化学物质组学联系起来，构建了药物-靶点-疾病之间的复杂网络。表2[17,54,55,63-70]和表3[71-76]分别总结了近年运用代谢组学及网络药理学筛选中药抗药源性肝损伤活性物质的相关研究。

2.2　内源性效应物质

中药的内源性效应物质包括肠道益生菌及内源性代谢产物，是人体内固有且具有肝保护活性的一类物质，可被外源活性物质及其代谢物调控，在体内直接介导药效发挥。内源性效应物质是中药抗药源性肝损伤效应物质的重要组成部分，也是药源性肝损伤治疗的关键潜在靶点。

2.2.1　肠道益生菌　肠道益生菌在抗药源性肝损伤发挥重要作用，肠道益生菌可以通过发酵产生代谢物或改变内源性物质代谢发挥作用。表4[77-96]对近年发现

Table 2 Applications of metabolomics in the screening of TCM against drug-induced liver injury

Research subject	Analytical technique	Result	Ref.
Protection by ginseng saponins against cyclophosphamide-induced liver injuries	LC-MS	14 potential biomarkers in serum samples were identified. Four pathways, including *L*-arginine and proline metabolism, phenylalanine metabolism, fatty acid biosynthesis, and valine, leucine and isoleucine biosynthesis, were all responsible for the regulation of liver injuries induced by cyclophosphamide treatment.	[17]
Ginseng alleviates cyclophosphamide-induced hepatotoxicity	UPLC-Q-TOF-MS/MS	19 endogenous biomarkers in serum samples were identified. The mechanism may be related to modulating the disordered homeostasis of primary bile acid and GSH *in vivo*.	[54]
Protective mechanism of astragalus polysaccharides against cantharidin-induced liver injury	LC-MS	23 differential metabolites enriched were identified. The mechanism may be related to regulating primary bile acid biosynthesis, glycerophospholipid metabolism.	[55]
Protective effect of Zheng Chaihu Yin on acetaminophen-induced acute liver injury	UPLC-Q-TOF-MS	19 potential biomarkers were identified. The mechanism may be related to the endogenous metabolites including lipid metabolism, amino acid metabolism, glucose metabolism and energy metabolism.	[63]
Protective effect of *Coptis chinensis* and berberine on cinnabar-induced hepatotoxicity	^{1}H NMR	14 potential biomarkers in urine and 9 biomarkers in serum samples were identified. The mechanism may be related to the endogenous metabolites including energy metabolism, amino acid metabolism and metabolism of intestinal flora in rats.	[64]
Hepatoprotective effect of *Citrus aurantium* L. against acetaminophen-induced liver injury	UPLC-Q-TOF-MS	44 endogenous biomarkers in serum samples were identified. The mechanism may be related to regulating liver metabolic disorders in glycerophospholipid metabolism, fatty acid biosynthesis and glycerolipid metabolism.	[65]
Green tea extract alleviates acetaminophen-induced hepatotoxicity	UPLC-Q-TOF-MS, NMR	Significantly altered pathways included fatty acid metabolism, glycerophospholipid metabolism, glutathione metabolism, and energy pathways.	[66]
Determine the mechanism underlying the effects of *Sagittaria sagittifolia* Polysaccharide on isoniazid- and rifampicin-induced hepatotoxicity	UPLC-HRMS	14 significantly differential metabolites were identified. The mechanism may be related to restoring fatty acid metabolism, taurine and hypotaurine metabolism, amino acid metabolism, the tricarboxylic acid cycle, and the ornithine cycle.	[67]
Protective function of *Schisandra* Lignans against acetaminophen-induced hepatotoxicity	GC-MS	35 kinds of differential small metabolites were identified. The most significant changes were in the urea cycle, ammonia recycling and arginine and proline metabolism.	[68]
Protection of paeonol against epirubicin-induced hepatotoxicity	GC-MS	7 endogenous metabolites were identified as the candidates of the potential biomarkers. The underlying metabolomic mechanisms refer to lipid metabolism, amino acid metabolism, energy metabolism and the AMPK/mTOR signaling pathway.	[69]
Indicating glycyrrhizin's protection against acetaminophen-induced liver damage	UFLC-triple TOF-MS	Targeted metabolomics study indicated that glycyrrhizin acts by reversing fatty acid metabolism.	[70]

具有药源性肝损伤肝保护作用的肠道益生菌进行归纳。这提示着具有抗药源性肝损伤作用的肠道菌可作为未来的治疗靶点, 补充益生菌或菌群移植来改善菌群结构是治疗或预防药源性肝损伤的可行之策。

近年逐步兴起的高通量测序技术为抗药源性肝损伤肠道益生菌的研究提供便利。高通量测序技术可以快速采集分析肠道菌群相关信息, 使检测不可培养物种成为可能。16S rRNA测序主要用于表征微生物群落丰度、多样性与结构特性, 检测优势种群和稀有种群, 并将相关肠道菌鉴定至属水平, 在表征生理与病理状态下菌群组成、丰度、结构特征差异等方面应用广泛。宏基因组在获取微生物群多样性、种群结构的基础上, 可进一步对其基因构成、代谢途径等进行分析, 探究基因功能、参与通路与相互作用关系, 且可以检测痕量微生物, 将肠道菌进一步鉴定至种水平[97], 这为筛选鉴定有抗肝损伤活性的益生菌提供了强有力的技术支撑。

Hong等[77]研究冬凌草甲素通过肠道菌群相关的方式改变尿素循环并减轻APAP致肝损伤。16S rRNA测序发现冬凌草甲素给药可转变肠道菌群结构, 在科水平上, 增加了*Akkermansiaceae*和*Bacteroidaceae*的丰度; 在属水平上, 增加了*Akkermansia*和*Bacteroides*的丰度, *B. vulgatus*在种水平上被显著富集, 且该菌丰度与血清ALT、AST水平密切相关。将*B. vulgatus*单独给小鼠灌胃给药发现, 小鼠血清ALT、AST水平降低, GSH、SOD水平上调, 肝损伤区域变小, 表明*B. vulgatus*可以抵抗APAP致肝损伤。进一步研究表明*B. vulgatu*通过尿素循环-Nrf2途径减弱APAP肝毒性, 提

Table 3 Application of network pharmacology in the screening of TCM against drug-induced liver injury

Category	Research subject	Database	Result	Ref.
Traditional Chinese medicine formulae	Hugan Tablets alleviate atorvastatin-induced hepatotoxicity	SciFinder, ChemSpider, UNIFI^{TM}, Mass Bank, SwissTargetPrediction, TCMSP, Online Mendelian Inheritance in Man, GeneCards database, UniProtKB, DAVID, STRING database, Cytoscape v3.7.1, RCSB, Discovery Studio 4.0 (DS) LibDock	Predicted 10 key targets, 19 active ingredients, and 5 signaling pathways including PI3K/Akt, TNF signaling pathway and others.	[71]
	Potential mechanism of Erzhi Pill against drug-induced liver injury	TCMSP, PharmMapper, SwissTargetPrediction, PubChem Compound database, DrugBank, GeneCards, UniProt, DAVID, STRING database, GO, KEGG	Predicted 10 key targets, 23 active ingredients, and 20 signaling pathways including PI3K/Akt, MAPK signaling pathway and others.	[72]
Traditional Chinese medicine	*Sedum sarmentosum* Bunge attenuates acetaminophen-induced liver injury	TCMSP, Cytoscape 3.7.1, HIT database, SysDTmodels, UniProt database, DisGeNET database, DigSee database, OMIM database, DAVID, STRING database	Predicted 61 key targets, 5 active ingredients, and 20 possible signaling pathways including MAPK, PI3K-Akt signaling pathway and others.	[73]
	Potential mechanism of Schisandrae Chinensis Fructus against acetaminophen-induced liver injury	OMIM database, DrugBank database, DiGSeE, PharmMapper Server, Uniprot, Cytoscape 3.6.1, BIOGRID, BIND, INTACT, MINT, GO, KEGG, Omicshare	Predicted 14 core targets, 17 active ingredients, and 15 possible signaling pathways including ErbB, PI3K/Akt signaling pathway and others.	[74]
Traditional Chinese medicine monomers	Hepatoprotective effect of forsythiaside A against acetaminophen-induced liver injury	GeneCards, Disgenet, Drugbank, OMIM database, Uniprot database, PubChem database, PharmMapper, SwissTargetPrediction, TCMSP, Drugbank database, DAVID 6.8, STRING database, Cytoscape v3.7.1, Bisogenet, CytoNCA	Predicted 17 core targets, and 65 possible signaling pathways including proteoglycans in cancer, pathways in cancer and others.	[75]
	Schisandrol A attenuates acetaminophen-induced liver injury	Swiss Target Prediction, Comparative Toxicogenomics database, STRING database, WebGestalt	Predicted 23 key targets, and 8 possible signaling pathways including TNF signaling pathway and others.	[76]

示*B. vulgatu*作为益生菌发挥抗肝损伤的治疗作用的潜力。

2.2.2 内源性代谢产物 目前, 中药抗药源性肝损伤的内源性代谢产物研究主要集中于由肠道菌群发酵产生的代谢物上。肠道菌群代谢物如短链脂肪酸 (short-chain fatty acids, SCFAs)、长链脂肪酸、肽类、氨基酸类等广泛参与并影响宿主的代谢和免疫系统, 可能对肝损伤具有积极作用[98]。

SCFAs参与维持肠道黏膜的完整性, 改善糖脂代谢, 控制能量消耗, 调节免疫系统和炎症反应, 减轻肝损伤。肠道菌群发酵产生的SCFAs中最丰富的是乙酸、丙酸和丁酸, 拟杆菌门可产生大量醋酸盐和丙酸盐, 厚壁菌门是丁酸盐的主要制造者。Huang等[57]通过非靶向代谢组学并结合进一步动物实验结果推测SCFAs给药可以改善TP引起的肝损伤, 且丙酸盐是发挥肝保护作用的主要原因。丙酸盐显著改善了TP引起的代谢紊乱, 改善肝脏损伤, 显著降低血清中转氨酶含量, 发挥肝保护作用。

色氨酸是一种必需的芳香族氨基酸, 是许多关键内源性效应物质的前体。饮食药物中所含色氨酸经由肠道菌群代谢, 代谢物如吲哚及其衍生物等通过激活芳烃受体/孕烷X受体保护肠道屏障和代谢稳定, 改善免疫反应, 减轻肝损伤。Wang等[99]研究灵仙新苷对TP诱导肝损伤的保护作用, 首先采用非靶向代谢组学推断出包括色氨酸代谢在内的三种主要代谢通路, 并进一步聚焦色氨酸代谢通路进行靶向代谢组学分析, 对血清及肝脏样本中色氨酸代谢相关物质进行定量。结果显示, 血清及肝脏中多种色氨酸代谢通路中相关衍生物如色氨酸、吡啶甲酸、吲哚甲酸、色胺等均有显著变化, 表明其作为生物标记物的潜力, 此外其生物学效应提示它们可能是治疗的潜在靶点, 可作为内源效应物质发挥抗肝损伤的治疗作用。

3 结语

药源性肝损伤发生的机制较为复杂, 但诸多研究表明药物并非通过单一机制引发肝损伤, 各种机制相互作用、相互影响。了解其发生发展的机制可以为进一步开发具有肝保护作用的临床药物提供方向。

随研究不断深入, 中药效应物质的内涵有了拓展。除传统意义上的中药活性成分及代谢物外, 内源性代谢产物和肠道益生菌在中药发挥药效过程中不可或缺, 与中药活性成分及代谢物一起构成“中药成分-肠道菌群-内源性效应物质”的完整体系, 三者相互关联。中药汤剂口服经胃肠道在肠道菌群作用下代谢, 以原形或者代谢物形式吸收入血, 作为外源性效应物质发挥肝保护作用; 肠道菌群结构受到中药成分影响而导致肠道微生物代谢物组发生改变, 影响肠道内源性物质的水平, 直接产生肝保护的药效活性。对中药效应物质的研究仍需进一步深入, 未来研究应着重三者联

Table 4　Summary of probiotics against drug-induced liver injury

Probiotics	Anti-liver injury mechanism	Related herbs with upregulating effect
Bacteroides		
Bacteroides vulgatus	Activate the urea cycle-Nrf2 pathway to regulate redox homeostasis against APAP[77]	Ginseng polysaccharides[78], *Folium senna* decoction[79]
Akkermansia		
Akkermansia muciniphila	Activate the PI3K/Akt pathway, regulate the composition and metabolic function of the intestinal microbiota, and alleviate oxidative stress and inflammation in the liver[80]	Rhubarb, *Ganoderma lucidum*, Ephedra sinica, Flos Lonicera, *Salvia miltiorrhiza* Bunge, *Codonopsis pilosula* Nannf, *Morinda officinalis*, *Astragalus membranaceus* and *Salvia miltiorrhiza*, Huang-Lian-Jie-Du-Decoction, Shenzhu Tiaopi Granule, Linggui Zhugan Formula, Tong-Xie-Yao-Fang, Wuji Wan Formula, puerarin[81]
Streptococcus		
Streptococcus salivarius subsp. thermophilus	Attenuate oxidative stress by decreasing the lipid peroxidation level and recovering antioxidant capacity[82]	Polysaccharide from Chinese Yam (*Dioscorea opposite* Thunb.)[83], soybean protein[84]
Lactobacillus		
Lactobacillus rhamnosus	Mitigate oxidative stress injury by activating the Nrf2 pathway[85]	Turmeric extract [86]
Lactobacillus acidophilus	Lysates significantly inhibit the APAP induced apoptosis, prevent mitochondrial damage, modulate crucial end points of oxidative stress induced apoptosis[87]	Polysaccharide obtained from persimmon (*Diospyros kaki* L.)[88], *Lycium barbarum* polysaccharide[89], evodiamine[90]
Lactobacillus fermentum	*Lactobacillus fermentum* postbiotic protects against APAP-induced HepG2 cytotoxicity by enhancing the activation of the PINK1 signaling pathway-dependent autophagy[91]	Polysaccharide from seeds of *Plantago asiatica* L.[92]
Lactobacillus casei	Increase the hepatic expression of HO-1, SOD2 and Bcl-2, alleviate oxidative stress, reduce inflammation, inhibit apoptosis[93]	Galactoglucan isolated from *Cistanche deserticola* Y. C. Ma.[94]
Bacillus		
Bacillus licheniformis *Bacillus indicus* *Bacillus subtilis* *Bacillus clausii* *Bacillus coagulans*	Probiotic blend of spores from five *Bacillus* species can alleviate oxidative stress and reduce inflammation[95]	*Bacillus coagulans* can be up-regulated by Sini Decoction[96]

系, 构建完整的中药抗药源性肝损伤的效应物质体系。

中药作为中华医药宝库中的重要组成部分, 在抗药源性肝损伤方面具有广泛的应用前景。但中药有效成分成分众多且尚不清楚, 作用机制复杂, 明确体内外中药效应物质仍然具有重要意义及广泛发展前景。研究过程需始终以中医药理论为指导, 体现中药及中药方剂作用“多成分、多靶点”的特性, 与现代理论、检测技术密切结合, 进行更为深入系统的挖掘, 阐释其物质基础与作用机制。

作者贡献: 黄心慧负责文献调研和文章撰写; 周婷婷负责文章选题、指导写作及文章审阅。

利益冲突: 本文作者均声明无利益冲突。

References

[1] Ntamo Y, Ziqubu K, Chellan N, et al. Drug-induced liver injury: clinical evidence of *N*-acetyl cysteine protective effects [J]. Oxid Med Cell Longev, 2021, 2021: 3320325.

[2] Zhang HY, Wang HL, Zhong GY, et al. Molecular mechanism and research progress on pharmacology of traditional Chinese medicine in liver injury [J]. Pharm Biol, 2018, 56: 594-611.

[3] Wang YX, Du Y, Liu XF, et al. A hepatoprotection study of Radix Bupleuri on acetaminophen-induced liver injury based on CYP450 inhibition [J]. Chin J Nat Med, 2019, 17: 517-524.

[4] Mahmoud AM, Hozayen WG, Ramadan SM. Berberine ameliorates methotrexate-induced liver injury by activating Nrf2/HO-1 pathway and PPARgamma, and suppressing oxidative stress and apoptosis in rats [J]. Biomed Pharmacother, 2017, 94: 280-291.

[5] Mahmoud AM, Hussein OE, Hozayen WG, et al. Ferulic acid prevents oxidative stress, inflammation, and liver injury *via* upregulation of Nrf2/HO-1 signaling in methotrexate-induced rats [J]. Environ Sci Pollut Res Int, 2020, 27: 7910-7921.

[6] Luo Y, Zhang X, Zhang W, et al. Compatibility with Semen Sojae Praeparatum attenuates hepatotoxicity of Gardeniae Fructus by regulating the microbiota, promoting butyrate production and activating antioxidant response [J]. Phytomedicine, 2021, 90: 153656.

[7] Sun D, Sun C, Qiu G, et al. Allicin mitigates hepatic injury following cyclophosphamide administration *via* activation of Nrf2/ARE pathways and through inhibition of inflammatory and apoptotic machinery [J]. Environ Sci Pollut Res Int, 2021, 28:

39625-39636.

[8] Cao LJ, Hou ZY, Li HD, et al. The ethanol extract of licorice (*Glycyrrhiza uralensis*) protects against triptolide-induced oxidative stress through activation of Nrf2 [J]. Evid Based Complement Alternat Med, 2017, 2017: 2752389.

[9] Wang Z, Ma J, Yao S, et al. Liquorice extract and 18beta-glycyrrhetinic acid protect against experimental pyrrolizidine alkaloid-induced hepatotoxicity in rats through inhibiting cytochrome P450-mediated metabolic activation [J]. Front Pharmacol, 2022, 13: 850859.

[10] Yang G, Zhang L, Ma L, et al. Glycyrrhetinic acid prevents acetaminophen-induced acute liver injury *via* the inhibition of CYP2E1 expression and HMGB1-TLR4 signal activation in mice [J]. Int Immunopharmacol, 2017, 50: 186-193.

[11] Fan S, Huang X, Wang S, et al. Combinatorial usage of fungal polysaccharides from Cordyceps sinensis and Ganoderma atrum ameliorate drug-induced liver injury in mice [J]. Food Chem Toxicol, 2018, 119: 66-72.

[12] Temel Y, Kucukler S, Yildirim S, et al. Protective effect of chrysin on cyclophosphamide-induced hepatotoxicity and nephrotoxicity *via* the inhibition of oxidative stress, inflammation, and apoptosis [J]. Naunyn Schmiedebergs Arch Pharmacol, 2020, 393: 325-337.

[13] Zhao Z, Wei Q, Hua W, et al. Hepatoprotective effects of berberine on acetaminophen-induced hepatotoxicity in mice [J]. Biomed Pharmacother, 2018, 103: 1319-1326.

[14] Tian S, Jiang X, Tang Y, et al. Laminaria japonica fucoidan ameliorates cyclophosphamide-induced liver and kidney injury possibly by regulating Nrf2/HO-1 and TLR4/NF-kappaB signaling pathways [J]. J Sci Food Agric, 2022, 102: 2604-2612.

[15] Aladaileh SH, Abukhalil MH, Saghir SAM, et al. Galangin activates Nrf2 signaling and attenuates oxidative damage, inflammation, and apoptosis in a rat model of cyclophosphamide-induced hepatotoxicity [J]. Biomolecules, 2019, 9: 346.

[16] Esmaeilzadeh M, Heidarian E, Shaghaghi M, et al. Gallic acid mitigates diclofenac-induced liver toxicity by modulating oxidative stress and suppressing IL-1beta gene expression in male rats [J]. Pharm Biol, 2020, 58: 590-596.

[17] Chen J, Li Z, Hua M, et al. Protection by ginseng saponins against cyclophosphamide-induced liver injuries in rats by induction of cytochrome P450 expression and mediation of the l-arginine/nitric oxide pathway based on metabolomics [J]. Phytother Res, 2021, 35: 3130-3144.

[18] Hassan RA, Hozayen WG, Abo Sree HT, et al. Naringin and hesperidin counteract diclofenac-induced hepatotoxicity in male wistar rats *via* their antioxidant, anti-inflammatory, and antiapoptotic activities [J]. Oxid Med Cell Longev, 2021, 2021: 9990091.

[19] Dogra A, Gupta D, Bag S, et al. Glabridin ameliorates methotrexate-induced liver injury *via* attenuation of oxidative stress, inflammation, and apoptosis [J]. Life Sci, 2021, 278: 119583.

[20] Caglayan C, Temel Y, Kandemir FM, et al. Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage [J]. Environ Sci Pollut Res Int, 2018, 25: 20968-20984.

[21] Heidarian E, Nouri A. Hepatoprotective effects of silymarin against diclofenac-induced liver toxicity in male rats based on biochemical parameters and histological study [J]. Arch Physiol Biochem, 2021, 127: 112-118.

[22] Wang L, Huang QH, Li YX, et al. Protective effects of silymarin on triptolide-induced acute hepatotoxicity in rats [J]. Mol Med Rep, 2018, 17: 789-800.

[23] Liu A, Tanaka N, Sun L, et al. Saikosaponin d protects against acetaminophen-induced hepatotoxicity by inhibiting NF-kappaB and STAT3 signaling [J]. Chem Biol Interact, 2014, 223: 80-86.

[24] Sherif IO. The effect of natural antioxidants in cyclophosphamide-induced hepatotoxicity: role of Nrf2/HO-1 pathway [J]. Int Immunopharmacol, 2018, 61: 29-36.

[25] Harchegani AB, Khor A, Niha MM, et al. The hepatoprotective and antioxidative effect of saffron stigma alcoholic extract against vincristine sulfate induced toxicity in rats [J]. Interdiscip Toxicol, 2019, 12: 186-191.

[26] Park G, Kim KM, Choi S, et al. Aconitum carmichaelii protects against acetaminophen-induced hepatotoxicity *via* B-cell lymphoma-2 protein-mediated inhibition of mitochondrial dysfunction [J]. Environ Toxicol Pharmacol, 2016, 42: 218-225.

[27] Lin G, Luo D, Liu J, et al. Hepatoprotective effect of polysaccharides isolated from *Dendrobium officinale* against acetaminophen-induced liver injury in mice *via* regulation of the Nrf2-Keap1 signaling pathway [J]. Oxid Med Cell Longev, 2018, 2018: 6962439.

[28] Shi L, Hao Z, Zhang S, et al. Baicalein and baicalin alleviate acetaminophen-induced liver injury by activating Nrf2 antioxidative pathway: the involvement of ERK1/2 and PKC [J]. Biochem Pharmacol, 2018, 150: 9-23.

[29] Ye XL, Jiang KY, Yang L, et al. The protective effect of salvianolic acid B against senecionine-induced hepatotoxicity in mice [J]. Acta Pharm Sin (药学学报), 2021, 56: 1079-1085.

[30] Xie W, Jiang Z, Wang J, et al. Protective effect of hyperoside against acetaminophen (APAP) induced liver injury through enhancement of APAP clearance [J]. Chem Biol Interact, 2016, 246: 11-19.

[31] Ci R, Zhang K, Zhu A, et al. Dendrobine attenuates isoniazid- and rifampicin-induced liver injury by inhibiting miR-295-5p [J]. Hum Exp Toxicol, 2020, 39: 1671-1680.

[32] Wu K, Fan J, Huang X, et al. Hepatoprotective effects exerted by *Poria cocos* polysaccharides against acetaminophen-induced liver injury in mice [J]. Int J Biol Macromol, 2018, 114: 137-142.

[33] Che J, Yang S, Qiao Z, et al. *Schisandra chinensis* acidic polysaccharide partialy reverses acetaminophen-induced liver injury

in mice [J]. J Pharmacol Sci, 2019, 140: 248-254.

[34] Che JY, Yang S, Qiao ZJ, et al. Effect of *Schisandra chinensis* polysaccharide on apoptosis of hepatocytes induced by acetaminophen in mice [J]. Sci Technol Food Ind (食品工业科技), 2019, 40: 285-289, 303.

[35] Leng J, Wang Z, Fu CL, et al. NF-kappaB and AMPK/PI3K/Akt signaling pathways are involved in the protective effects of Platycodon grandiflorum saponins against acetaminophen-induced acute hepatotoxicity in mice [J]. Phytother Res, 2018, 32: 2235-2246.

[36] Cai L, Zou S, Liang D, et al. Structural characterization, antioxidant and hepatoprotective activities of polysaccharides from *Sophorae tonkinensis* Radix [J]. Carbohydr Polym, 2018, 184: 354-365.

[37] Xu LQ, Zhou YY, Jiang YM, et al. Wuzhi Tablet protects against APAP-induced liver injury at different pretreated intervals in mice [J]. Acta Pharm Sin (药学学报), 2021, 56: 1147-1154.

[38] Chen S, Wu Q, Li X, et al. Characterization of cytochrome P450s (CYP)-overexpressing HepG2 cells for assessing drug and chemical-induced liver toxicity [J]. J Environ Sci Health C Toxicol Carcinog, 2021, 39: 68-86.

[39] Meurer L, Cohen SM. Drug-induced liver injury from statins [J]. Clin Liver Dis, 2020, 24: 107-119.

[40] Zheng N, Wei A, Wu T, et al. Triptolide and atorvastatin synergistically promote hepatotoxicity in cultured hepatocytes and female Sprague-Dawley rats by inhibiting pregnane X receptor-mediated transcriptional activation of CYP3A4 [J]. Toxicol Lett, 2021, 342: 85-94.

[41] Huo W, Li H, Li L, et al. Study on regulation of CYP450 enzyme system to reduce liver toxicity through compatibility of Aconiti Kusnezoffii Radix Cocta with Chebulae Fructus and Glycyrrhizae Radix et Rhizoma [J]. China J Chin Mater Med (中国中药杂志), 2022, 47: 1618-1624.

[42] Su LJ, Zhang JH, Gomez H, et al. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis [J]. Oxid Med Cell Longev, 2019, 2019: 5080843.

[43] Henninger C, Huelsenbeck J, Huelsenbeck S, et al. The lipid lowering drug lovastatin protects against doxorubicin-induced hepatotoxicity [J]. Toxicol Appl Pharmacol, 2012, 261: 66-73.

[44] Prasanna PL, Renu K, Valsala Gopalakrishnan A. New molecular and biochemical insights of doxorubicin-induced hepatotoxicity [J]. Life Sci, 2020, 250: 117599.

[45] Fromenty B. Alteration of mitochondrial DNA homeostasis in drug-induced liver injury [J]. Food Chem Toxicol, 2020, 135: 110916.

[46] Ramachandran A, Jaeschke H. Acetaminophen hepatotoxicity: a mitochondrial perspective [J]. Adv Pharmacol, 2019, 85: 195-219.

[47] Chen M, Suzuki A, Borlak J, et al. Drug-induced liver injury: interactions between drug properties and host factors [J]. J Hepatol, 2015, 63: 503-514.

[48] Jiang X, Li D, Si LQ, et al. Research progress in mechanisms of idiosyncratic drug-induced liver injury mediated by inflammatory stress [J]. Acta Pharm Sin (药学学报), 2021, 56: 1544-1550.

[49] Xu K, Liu G, Fu C. The tryptophan pathway targeting antioxidant capacity in the placenta [J]. Oxid Med Cell Longev, 2018, 2018: 1054797.

[50] Gao J, Shi N, Guo H, et al. UPLC-Q-TOF/MS-based metabolomics approach to reveal the hepatotoxicity of emodin and detoxification of dihydromyricetin [J]. ACS Omega, 2021, 6: 5348-5358.

[51] Metushi IG, Nakagawa T, Uetrecht J. Direct oxidation and covalent binding of isoniazid to rodent liver and human hepatic microsomes: humans are more like mice than rats [J]. Chem Res Toxicol, 2012, 25: 2567-2576.

[52] Metushi IG, Sanders C, The Acute Liver Study Group, et al. Detection of anti-isoniazid and anti-cytochrome P450 antibodies in patients with isoniazid-induced liver failure [J]. Hepatology, 2014, 59: 1084-1093.

[53] Tu C, Gao Y, Song D, et al. Screening for susceptibility-related biomarkers of diclofenac-induced liver injury in rats using metabolomics [J]. Front Pharmacol, 2021, 12: 693928.

[54] Zhu H, Long MH, Wu J, et al. Ginseng alleviates cyclophosphamide-induced hepatotoxicity *via* reversing disordered homeostasis of glutathione and bile acid [J]. Sci Rep, 2015, 5: 17536.

[55] Huang X, Tang W, Lin C, et al. Protective mechanism of *Astragalus* polysaccharides against cantharidin-induced liver injury determined in vivo by liquid chromatography/mass spectrometry metabolomics [J]. Basic Clin Pharmacol Toxicol, 2021, 129: 61-71.

[56] Wu G, Win S, Than TA, et al. Gut microbiota and liver injury (I)-acute liver injury [J]. Adv Exp Med Biol, 2020, 1238: 23-37.

[57] Huang JF, Zhao Q, Dai MY, et al. Gut microbiota protects from triptolide-induced hepatotoxicity: key role of propionate and its downstream signalling events [J]. Pharmacol Res, 2020, 155: 104752.

[58] Schneider KM, Elfers C, Ghallab A, et al. Intestinal dysbiosis amplifies acetaminophen-induced acute liver injury [J]. Cell Mol Gastroenterol Hepatol, 2021, 11: 909-933.

[59] Letertre MPM, Munjoma N, Wolfer K, et al. A two-way interaction between methotrexate and the gut microbiota of male Sprague-Dawley rats [J]. J Proteome Res, 2020, 19: 3326-3339.

[60] Yang XR, Dou X, Li GF, et al. Research progress on prevention and treatment of hepatotoxicity induced by *Gardenia jasminoides* [J]. Chin Tradit Herb Drugs (中草药), 2022, 53: 4170-4176.

[61] Chang R, Liu J, Luo Y, et al. Isoflavones' effects on pharmacokinetic profiles of main iridoids from Gardeniae Fructus in rats [J]. J Pharm Anal, 2020, 10: 571-580.

[62] Li F, Zhao Y, Lin R, et al. Research progress on the effective substances and the mechanism of traditional Chinese medicine com-

pound [J]. Chin Pharm J (中国药学杂志), 2019, 54: 1037-1044.

[63] Liu XF, Wu X, Yang FX, et al. Protective effect of Zheng Chaihu Yin on paracetamol induced acute liver injury of mice [J]. Chin J Pharmacol Toxicol (中国药理学与毒理学杂志), 2017, 31: 101-111.

[64] Su G, Wang H, Gao Y, et al. ^{1}H-NMR-based metabonomics of the protective effect of *Coptis chinensis* and berberine on cinnabar-induced hepatotoxicity and nephrotoxicity in rats [J]. Molecules, 2017, 22: 1855.

[65] Shu Y, He D, Li W, et al. Hepatoprotective effect of *Citrus aurantium* L. against APAP-induced liver injury by regulating liver lipid metabolism and apoptosis [J]. Int J Biol Sci, 2020, 16: 752-765.

[66] Lu Y, Sun J, Petrova K, et al. Metabolomics evaluation of the effects of green tea extract on acetaminophen-induced hepatotoxicity in mice [J]. Food Chem Toxicol, 2013, 62: 707-721.

[67] Ke XH, Wang CG, Luo WZ, et al. Metabolomic study to determine the mechanism underlying the effects of *Sagittaria sagittifolia* polysaccharide on isoniazid- and rifampicin-induced hepatotoxicity in mice [J]. Molecules, 2018, 23: 3087.

[68] Yan C, Guo H, Ding Q, et al. Multiomics profiling reveals protective function of *Schisandra* lignans against acetaminophen-induced hepatotoxicity [J]. Drug Metab Dispos, 2020, 48: 1092-103.

[69] Jing X, Sun C, Chen H, et al. Protection of paeonol against epirubicin-induced hepatotoxicity: a metabolomic study [J]. Biosci Trends, 2019, 13: 253-260.

[70] Yu J, Jiang YS, Jiang Y, et al. Targeted metabolomic study indicating glycyrrhizin's protection against acetaminophen-induced liver damage through reversing fatty acid metabolism [J]. Phytother Res, 2014, 28: 933-936.

[71] Lv S, Yu H, Liu X, et al. The study on the mechanism of Hugan tablets in treating drug-induced liver injury induced by atorvastatin [J]. Front Pharmacol, 2021, 12: 683707.

[72] Huang SJ, Mu F, Li F, et al. Systematic elucidation of the potential mechanism of Erzhi pill against drug-induced liver injury *via* network pharmacology approach [J]. Evid Based Complement Alternat Med, 2020, 2020: 6219432.

[73] Jiang Z, Han Y, Zhang Y, et al. *Sedum sarmentosum* Bunge attenuates drug-induced liver injury *via* Nrf2 signaling pathway: an experimental verification based on network pharmacology prediction [J]. J Healthc Eng, 2021, 2021: 1142638.

[74] Li X, Yang H, Xiao J, et al. Network pharmacology based investigation into the bioactive compounds and molecular mechanisms of Schisandrae Chinensis Fructus against drug-induced liver injury [J]. Bioorg Chem, 2020, 96: 103553.

[75] Gong L, Zhou H, Wang C, et al. Hepatoprotective effect of forsythiaside a against acetaminophen-induced liver injury in zebrafish: coupling network pharmacology with biochemical pharmacology [J]. J Ethnopharmacol, 2021, 271: 113890.

[76] Li X, Ge J, Li M, et al. Network pharmacology, molecular docking technology integrated with pharmacodynamic study to reveal the potential targets of schisandrol A in drug-induced liver injury by acetaminophen [J]. Bioorg Chem, 2022, 118: 105476.

[77] Hong MK, Liu HH, Chen GH, et al. Oridonin alters hepatic urea cycle *via* gut microbiota and protects against acetaminophen-induced liver injury [J]. Oxid Med Cell Longev, 2021, 2021: 3259238.

[78] Huang J, Liu D, Wang Y, et al. Ginseng polysaccharides alter the gut microbiota and kynurenine/tryptophan ratio, potentiating the antitumour effect of antiprogrammed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy [J]. Gut, 2022, 71: 734-745.

[79] Yuan Z, Zhang C, Peng X, et al. Intestinal microbiota characteristics of mice treated with *Folium senna* decoction gavage combined with restraint and tail pinch stress [J]. 3 Biotech, 2020, 10: 180.

[80] Xia J, Lv L, Liu B, et al. *Akkermansia muciniphila* ameliorates acetaminophen-induced liver injury by regulating gut microbial composition and metabolism [J]. Microbiol Spectr, 2022, 10: e0159621.

[81] Bu F, Zhang S, Duan Z, et al. A critical review on the relationship of herbal medicine, *Akkermansia muciniphila*, and human health [J]. Biomed Pharmacother, 2020, 128: 110352.

[82] Riane K, Ouled-Haddar H, Alyane M, et al. Assessment of *Streptococcus salivarius* sp thermophiles antioxidant efficiency and its role in reducing paracetamol hepatotoxicity [J]. Iran J Biotechnol, 2019, 17: e2061.

[83] Ouyang J, Wang F, Li W, et al. Structure characterization of polysaccharide from Chinese yam (*Dioscorea opposite* Thunb.) and its growth-promoting effects on *Streptococcus thermophilus* [J]. Foods, 2021, 10: 2698.

[84] Hongfei Z, Fengling B, Fang Z, et al. Characterization of soybean protein hydrolysates able to promote the proliferation of *Streptococcus thermophilus* ST [J]. J Food Sci, 2013, 78: M575-M581.

[85] Saeedi BJ, Liu KH, Owens JA, et al. Gut-resident *Lactobacilli* activate hepatic Nrf2 and protect against oxidative liver injury [J]. Cell Metab, 2020, 31: 956-968.

[86] Ghiamati Yazdi F, Soleimanian-Zad S, van den Worm E, et al. Turmeric extract: potential use as a prebiotic and anti-inflammatory compound? [J]. Plant Foods Hum Nutr, 74: 293-299.

[87] Sharma S, Singh RL, Kakkar P. Modulation of Bax/Bcl-2 and caspases by probiotics during acetaminophen induced apoptosis in primary hepatocytes [J]. Food Chem Toxicol, 2011, 49: 770-779.

[88] Yang Z, Xu M, Li Q, et al. The beneficial effects of polysaccharide obtained from persimmon (*Diospyros kaki* L.) on the proliferation of *Lactobacillus* and gut microbiota [J]. Int J Biol Macromol, 2021, 182: 1874-1882.

[89] Zhu W, Zhou S, Liu J, et al. Prebiotic, immuno-stimulating and gut microbiota-modulating effects of *Lycium barbarum* polysaccharide [J]. Biomed Pharmacother, 2020, 121: 109591.

[90] Wang MX, Lin L, Chen YD, et al. Evodiamine has therapeutic efficacy in ulcerative colitis by increasing *Lactobacillus acidophilus* levels and acetate production [J]. Pharmacol Res, 2020, 159: 104978.

[91] Dinic M, Lukic J, Djokic J, et al. *Lactobacillus fermentum* postbiotic-induced autophagy as potential approach for treatment of acetaminophen hepatotoxicity [J]. Front Microbiol, 2017, 8: 594.

[92] Hu JL, Nie SP, Wu QM, et al. Polysaccharide from seeds of *Plantago asiatica* L. affects lipid metabolism and colon microbiota of mouse [J]. J Agric Food Chem, 2014, 62: 229-234.

[93] Zhang XH, Yan Y, Zhao SM, et al. Protective effect of *Lactobacillus casei* Zhang against acetaminophen-induced acute liver injury in mice [J]. Chin J Immunol (中国免疫学杂志), 2017, 33: 1761-1764.

[94] Zeng H, Huang L, Zhou L, et al. A galactoglucan isolated from of *Cistanche deserticola* Y. C. Ma. and its bioactivity on intestinal bacteria strains [J]. Carbohydr Polym, 2019, 223: 115038.

[95] Neag MA, Catinean A, Muntean DM, et al. Probiotic *Bacillus* spores protect against acetaminophen induced acute liver injury in rats [J]. Nutrients, 2020, 12: 632.

[96] Wang Y, Zhang X, Li J, et al. Sini decoction ameliorates colorectal cancer and modulates the composition of gut microbiota in mice [J]. Front Pharmacol, 2021, 12: 609992.

[97] Wang XN, Wang WW, Xin GZ, et al. Research advances on the composition and metabolites of intestinal flora regulated by Chinese herbal medicine [J]. Acta Chin Med Pharmacol (中医药学报), 2019, 47: 117-124.

[98] Agus A, Clement K, Sokol H. Gut microbiota-derived metabolites as central regulators in metabolic disorders [J]. Gut, 2021, 70: 1174-1182.

[99] Wang XN, Xia WR, Liu JQ, et al. Targeting tryptophan metabolism reveals clematichinenoside AR alleviates triptolide-induced hepatotoxicity [J]. J Pharm Biomed Anal, 2022, 208: 114461.