PPARα-中长链酰基肉碱轴在肝损伤及中药保肝中的作用研究

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摘要: 本研究聚焦过氧化物酶体增殖物激活受体α (peroxisome proliferator-activated receptor alpha, PPARα) 调控的中长链酰基肉碱 (medium- and long-chain acylcarnitines, MCACs/LCACs) 代谢通路, 旨在探讨其在中药干预肝损伤中的作用机制。本研究通过数据库与文献挖掘, 分析PPARα激动剂在肝病中的应用现状及MCACs/LCACs蓄积在肝病发生发展中的作用。进一步结合文献证据与动物实验, 验证中药及其成分通过PPARα-MCACs/LCACs通路干预肝病的潜在机制。实验结果表明, 雷公藤甲素联合雷公藤红素 (TP_Cel) 可诱导肝损伤, 并伴随血浆MCACs/LCACs蓄积 ($P < 0.05$)。在TP_Cel模型中, Spearman相关性分析显示, 血浆MCACs/LCACs水平与肝脏PPARα靶基因 (*Cpt1a*、*Cpt2*、*Acox1*) 表达呈中度至高度负相关, 且多数相关性具有统计学意义 ($P < 0.05$)。此外, *Ppara*$^{-/-}$小鼠血浆中MCACs/LCACs水平较野生型129小鼠显著升高 ($P < 0.05$)。最后, 构建多阶段筛选流程, 获得68种靶向PPARα、无明确肝毒性记录的保肝中药 (hepatoprotective herbal medicines, HpHMs) 及29种保肝成分 (hepatoprotective herbal ingredients, HpHIs)。分子对接初步验证了HpHIs与PPARα的结合能力, 为中药靶向脂质代谢紊乱相关肝病的机制研究与候选药物开发提供理论依据和潜在资源。动物实验方案经四川大学华西医院动物实验伦理委员会批准 (批准号: 20220217001)。

关键词: 过氧化物酶体增殖物激活受体α; 中长链酰基肉碱; 脂肪酸氧化障碍; 肉碱棕榈酰转移酶; 肝损伤; 中药筛选; 代谢组学

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Investigation of the role of the PPARα-medium/long-chain acylcarnitines axis in liver injury and the hepatoprotective effects of herbal medicines

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Abstract: This study focused on the medium- and long-chain acylcarnitines (MCACs/LCACs) metabolic axis regulated by peroxisome proliferator-activated receptor alpha (PPARα), aiming to elucidate its potential role in the hepatoprotective effects of herbal medicines. Initially, literature mining and database analysis were conducted to summarize the current research applications of PPARα agonists in liver diseases and the pathological implications

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of MCACs/LCACs accumulation across different liver disease models. Furthermore, published studies and animal experiments were integrated to validate the potential mechanism by which herbal medicines and herbal ingredients modulate liver disease *via* the PPARα-MCACs/LCACs pathway. Experimental results showed that co-administration of triptolide and celastrol (TP_Cel) significantly induced liver injury, accompanied by marked accumulation of MCACs/LCACs. In the TP_Cel model, Spearman analysis revealed moderately to strongly inverse correlations between plasma MCACs/LCACs abundances and the expression of hepatic PPARα-target genes, including *Cpt1a*, *Cpt2*, and *Acox1*, with most correlations reaching statistical significance ($P < 0.05$). In addition, plasma MCACs/LCACs abundances were significantly elevated in *Ppara*$^{-/-}$ mice compared with wild-type 129 mice ($P < 0.05$). Based on these findings, a multi-stage screening strategy was established, resulting in the identification of 68 hepatoprotective herbal medicines (HpHMs) and 29 hepatoprotective herbal ingredients (HpHIs), both targeting PPARα with no documented hepatotoxicity. Finally, molecular docking was employed to preliminarily evaluate the binding capacity of HpHIs to PPARα. These findings provide mechanistic insights and potential resources for the development of herbal medicines and herbal ingredients targeting lipid metabolism disorders in liver diseases. All animal experiments were approved by the Animal Ethics Committee of West China Hospital, Sichuan University (approval No. 20220217001).

Key words: peroxisome proliferator-activated receptor alpha; medium- and long-chain acylcarnitine; impaired fatty acid oxidation; carnitine palmitoyltransferase; liver injury; herbal medicine screening; metabolomics

脂质代谢紊乱是多种肝病的重要病理特征, C6～C20中长链酰基肉碱 (medium- and long-chain acylcarnitines, MCACs/LCACs) 是脂肪酸氧化受阻时积聚的重要代谢产物, 可反映线粒体脂质代谢紊乱及功能损伤状态[1,2]。正常情况下, 中长链脂肪酸经肉碱棕榈酰转移酶1 (carnitine palmitoyltransferase 1, CPT1) 催化形成MCACs/LCACs, 通过肉碱–酰基肉碱转位酶 (carnitine-acylcarnitine translocase, CACT) 进入线粒体, 再经肉碱棕榈酰转移酶2 (carnitine palmitoyltransferase 2, CPT2) 转化为酰基辅酶A进入β-氧化。当该过程受阻时, MCACs/LCACs在肝细胞内蓄积, 诱发肝肿大、功能障碍及脂肪变性, 从而加重肝损伤。临床研究发现, MCACs/LCACs异常升高与代谢功能障碍相关脂肪性肝病 (metabolic dysfunction-associated steatotic liver disease, MASLD)[3]、药物性肝损伤 (drug induced liver injury, DILI)[4]、酒精性肝病 (alcohol associated liver disease, ALD)[5]及肝癌[6]等多种肝病的发生发展关系密切。

过氧化物酶体增殖物激活受体α (peroxisome proliferator-activated receptor alpha, PPARα) 是调控脂肪酸代谢的重要核转录因子, 可通过激活*CPT1*[7]、*CACT*[8]和*CPT2*[9]基因表达, 促进MCACs/LCACs的代谢清除[4], 缓解脂质代谢紊乱和肝损伤。目前, 以非诺贝特、佩玛贝特等为代表的合成PPARα激动剂已在临床上用于MASLD[10]、胆汁淤积性肝病[11]等肝病治疗, 但长期使用存在不良反应及个体差异, 临床应用受到一定限制。

近年来, 中药及其活性成分因多靶点调控、安全性好、临床基础广泛, 逐渐受到关注。中药成分如柠檬苦素[12]等可上调PPARα促进脂肪酸氧化而减轻肝损伤。但中药能否通过PPARα-MCACs/LCACs代谢通路干预肝脂代谢障碍并发挥保肝作用, 尚缺乏系统性研究支持。

基于上述背景, 本研究聚焦PPARα调控的MCACs/LCACs代谢通路, 系统梳理PPARα激动剂在肝病中的应用现状及MCACs/LCACs蓄积与肝损伤之间的病理关联; 结合中药文献研究与肝损伤动物模型, 验证中药通过PPARα-MCACs/LCACs代谢通路干预肝病的潜在作用; 最后, 整合多数据库筛选具有PPARα激动潜力且无肝毒性风险的保肝中药 (hepatoprotective herbal medicines, HpHMs) 及保肝成分 (hepatoprotective herbal ingredients, HpHIs), 并以分子对接初步探讨其与PPARα的结合能力与结构特征, 为中药干预脂质代谢紊乱相关肝病提供理论依据与候选资源。

材料与方法

仪器 超高效液相色谱串联傅里叶变换质谱 (UHPLC-Q Exactive Plus, Thermo Fisher, 美国); HSS T3色谱柱 (100 mm × 2.1 mm, 1.8 μm, Waters, 美国); 酶标板恒温震荡器 (MB100-2A, 杭州奥盛); 荧光定量qPCR仪 (CFX Connect, BIO-RAD, 美国)。

药品与试剂 雷公藤甲素 (triptolide, TP, 货号A0104) 与雷公藤红素 (celastrol, Cel, 货号A0106) 购自成都明斯特生物技术有限公司, 纯度均大于98%。丙氨酸氨基转移酶 (alanine aminotransferase, ALT, 货号C009-3-1)、天门冬氨酸氨基转移酶 (aspartate aminotransferase, AST, 货号C010-3-1) 与总胆汁酸

(total bile acid, TBA, 货号 E003-1-1) 检测试剂盒购自南京建成生物工程研究所。RNA 提取使用南京诺唯赞生物科技有限公司的 TRIzol 试剂 (货号 R411-01), qPCR 引物由生工生物工程 (上海) 股份有限公司合成。

动物实验设计与样本采集 ① 肝损伤模型: 6 周龄雄性 SPF 级 C57BL/6J 小鼠 [购自江苏集萃药康生物科技股份有限公司, 许可证号: SCXK (苏) 2023-0009] 饲养于清洁级动物房 (22 ± 1 ℃、40%～65% 湿度、12 h 光/暗循环), 自由摄食饮水。适应性喂养 5 天后, 随机分为 CON 组和 TP_Cel 组 (n = 4)。TP_Cel 组灌胃给予 1 mg·kg^{-1} TP 与 10 mg·kg^{-1} Cel 联合制剂 (溶剂: 1% 二甲基亚砜, 2% Tween 80, 97% 生理盐水)[13]; CON 组给予等量溶剂。24 h 后处死, 采集血浆与肝组织。② PPARα 敲除模型: 选用实验室自繁殖的 6 周龄雄性 129/Sv 背景 *Ppara*$^{-/-}$ 小鼠及其同背景野生型 (WT-129) 小鼠 (n = 4)[14]。小鼠于相同条件下适应性喂养 5 天后处死, 采集血浆。所有动物实验方案经四川大学华西医院动物实验伦理委员会批准 (批准号: 20220217001)。

生化、组织学、分子代谢组学与统计学分析 血浆样本经 4 ℃、2 000 ×g 离心 5 min 取上清, 酶法试剂盒检测 ALT、AST、TBA 水平。肝组织经 10% 缓冲福尔马林固定、常规脱水、透明、石蜡包埋制备 4 μm 切片, 苏木精−伊红 (hematoxylin and eosin, HE) 染色观察组织病理变化。TRIzol 法提取肝组织总 RNA, 反转录为 cDNA 后进行 qPCR, 以 *Gapdh* 为内参, $2^{-\Delta\Delta Ct}$ 法计算基因 (*Ppara*、*Cpt1a*、*Cpt2*、*Acox1*、*Cyp4a10*) 相对表达量 (引物见表 1)。血浆非靶向代谢组学分析基于本实验室既有方法与数据库进行[14,15], Xcalibur 软件 (Thermo Fisher) 手动积分提取 MCACs/LCACs 相对峰面积。数据为均值 ± 标准差, 组间比较依据分布选用 t 检验、Welch's 或 Mann-Whitney U 检验; Spearman 法进行相关性分析 [变量经 lg(x + 1) 转换和 Z-score 标准化], P < 0.05 为差异显著。

PPARα 激动剂的筛选与保肝作用评估 通过 DrugBank 检索 PPARα 靶向药物, 排除抑制剂、调节剂、激活剂、作用机制不明及已撤市药物; 结合 PubMed 系统检索各药物与“liver injury/hepatoprotective/hepatic”等肝病关键词共现文献, 剔除综述等二次文献及严重肝毒性风险药物, 使用 Cytoscape 3.8.2 构建药物−肝病关联网络, 评估 PPARα 激动剂的保肝潜力。

MCACs/LCACs 与肝病表型关系挖掘 通过比较毒理基因组学数据库 (comparative toxicogenomics database, CTD) 检索酰基肉碱 (acylcarnitine) 与肝病的相关性 (记录 inference score)。结合 PubMed 系统检索“acylcarnitine”及肝病关键词, 筛选原始研究中 MCACs/LCACs 蓄积的肝病类型及表型, 通过和弦图可视化共现关系。

PPARα 靶向保肝中药及保肝成分筛选流程 HpHMs 定义为靶向 PPARα、无肝毒性且应用于保肝方剂的中药; HpHIs 为来源于 HpHMs 的靶向 PPARα 且无肝毒性的活性成分。通过多数据库[16-20]筛选靶向 PPARα 的中药及其成分, 结合文献剔除具有毒性风险的条目, 获得候选 HpHMs 与 HpHIs; 随后分别与保肝方剂的组成中药及 HpHMs 的活性成分信息取交集, 确定最终 HpHMs 与 HpHIs, 构建相应关联网络。筛选流程见图 1。

保肝成分与 PPARα 蛋白分子对接 通过分子对接评估筛选的 HpHIs 与 PPARα 蛋白的结合能力。小分子配体和蛋白受体 (PDB id: 6KAZ) 的结构分别从 TCMSP 和 PDB 数据库中获得, 利用 SwissDock 在线平台在 6KAZ 蛋白的活性口袋中 (x = 9, y = 6, z = −7) 进行柔性对接[21,22], 结合构象由 PyMOL 2.4.1 和 Discovery Studio Visualizer 进行可视化。HpHIs 化学类型由 NP Classifier 分类注释[23]。

结果

1 PPARα 激动剂在肝病治疗中的应用现状

在 DrugBank 数据库中共筛选出 12 种具有 PPARα 激动活性的临床化合物, 文献统计显示这些激动剂已在 27 类肝病研究中应用 (图 2; 橙色−激动剂, 绿色−肝病, 绿色深浅−药物数, 线条粗细−文献数), 涵盖代谢性、毒性、胆汁淤积性、免疫性及肿瘤性肝损伤。其中, 非诺贝特为最常用于研究的代表性药物, 天然成分紫檀芪的适应证分布与之相似, 都在 MASLD、DILI、ALD 等多种肝病中应用, 而没药主要用于肝癌、高氨血症和血吸虫病模型。

Table 1 Primer sequences used for qPCR analysis

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
Gapdh	TTGTATGTGCAACAATCTCCAC	CGTCCCGTAGACAAAATGTGT
Ppara	CCCAAGGGAGGAATAGCTTCT	CTCTGCGATGCGGTTCCAA
Cpt1a	TGGCATCATCACTGGTGTGTT	CTCTAGGGTCCGATTGATCTTTG
Cpt2	CAGCACAGCATCGTACCCA	TCCCAATGCCGTTCTCAAAAT
Acox1	CCGCCACCTTCAATCCAGAG	CAAGTTCTCGATTTCTCGACGG
Cyp4a10	CCAGGAACTGCATTGGGAAA	GACCCTGGTAGGATCTGGCA

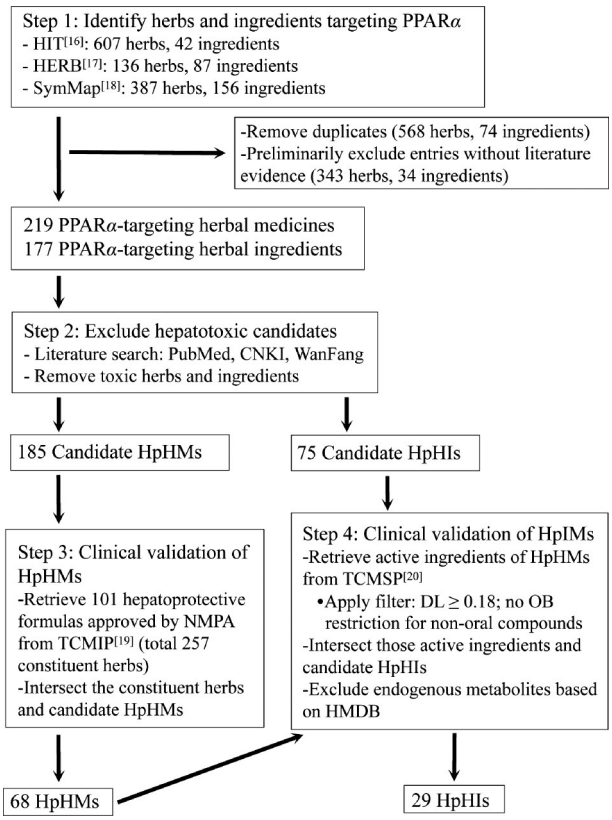


Figure 1 Flow diagram for the screening of hepatoprotective herbal medicines (HpHMs) and hepatoprotective herbal ingredients (HpHIs). PPARα: Peroxisome proliferator-activated receptor alpha; CNKI: China National Knowledge Infrastructure; NMPA: National Medical Products Administration; TCMIP[19]: Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine; TCMSP[20]: Traditional Chinese Medicine Systems Pharmacology; DL: Drug-likeness; OB: Oral bioavailability; HMDB: Human metabolome database; HIT[16]: Herbal Ingredients' Targets Platform; SymMap[18]: Symptom Mapping; HERB[17]: A high-throughput experiment- and reference-guided database of traditional Chinese medicine

2 MCACs/LCACs蓄积在肝病中的关联特征与机制线索

鉴于MCACs/LCACs为脂肪酸氧化通路的中间产物, 且其代谢可能受PPARα调控, 本研究探讨MCACs/LCACs蓄积在肝损伤中的潜在作用。CTD数据库预测分析显示, 酰基肉碱代谢异常与多种肝病高度相关, 包括高精氨酸血症、脂肪肝、DILI及肝癌等 (图3A)。

和弦图展示了文献中MCACs/LCACs与多种肝病及相关表型的共现关系 (图3B), 其升高广泛见于多类肝病, 包括代谢性、毒性、胆汁淤积性、氧化应激相关、肿瘤性及终末期肝病, 尤以DILI和MASLD最为常见。此外, MCACs/LCACs也常见于CACT、CPT2缺陷等脂肪酸氧化障碍相关的遗传性疾病。相关肝病表型包括肝脂肪变性、胆汁淤积、转氨酶升高、能量代谢紊乱及炎症等, MCACs/LCACs升高与脂肪酸β-氧化障碍、脂肪变性和线粒体功能障碍密切共现, 提示MCACs/LCACs代谢异常可能为脂肪酸氧化障碍型肝损伤的重要介质及潜在标志物。

3 PPARα通路介导的MCACs/LCACs代谢调控在肝损伤中的作用

3.1 文献证据支持PPARα-MCACs/LCACs-肝病机制 PPARα可能通过调节MCACs/LCACs来干预肝损伤, 而*CPT1/2*是其中介导的重要基因。文献调研发现, 赤芍、肉豆蔻、五酯片等中药及中成药在上调PPARα表达、缓解肝损伤的同时, 常伴有MCACs/LCACs水平的下降及*CPT1/2*表达的上调。相反, 在TP诱导的肝损伤模型中, PPARα表达受抑, *CPT1/2*下调并伴随MCACs/LCACs蓄积。此外, 在人参皂苷Rg1、水飞蓟宾、何首乌等多种中药及成分干预MASLD等肝病的研究中, 亦观察到PPARα与CPT1/2协同上调的现象 (表2[24-35])。这些结果表明, CPT1/2可能在PPARα-MCACs/LCACs-肝病机制中起着关键的介导作用。

3.2 TP与Cel联合肝损伤模型中PPARα抑制与MCACs/LCACs蓄积关联 TP和Cel为雷公藤制剂的主要活性成分, 联合用药可加重肝损伤[13]。因此, 本研究建立TP_Cel联合诱导的肝损伤模型, 探讨PPARα下调对CPT1/2及MCACs/LCACs代谢通路的影响。

Table 2 Herbal medicines and herbal ingredients modulating CPT1/2 and MCACs/LCACs *via* PPARα to improve liver disease

Herbal medicine/ingredient	PPARα-MCACs/LCACs axis alteration	Hepatic impact
Paeoniae radix rubra[24]	PPARα ↑, MCACs/LCACs ↓	Protect cholestatic model
Triptolide[25]	PPARα ↓, CPT1/2 ↓, MCACs/LCACs ↑	Damage
Nutmeg[26]	PPARα ↑, MCACs/LCACs ↓	Protect DILI
Ginsenoside Rg1[27]	PPARα ↑, CPT1/2 ↑	Protect MASLD
Wuzhi tablet[28]	PPARα ↑, CPT1 ↑, MCACs/LCACs ↓	Protect DILI
Silybin[29]	PPARα ↑, CPT1/2 ↑	Protect MASLD
Swertia cincta[30]	PPARα ↑, CPT2 ↑	Protect cholestatic model
Dendrobine[31]	PPARα ↑, CPT2 ↑	Protect MASLD
Tangerine peel derived exosome like nanovesicles[32]	PPARα ↑, CPT1/2 ↑	Protect MASLD
Monoammonium glycyrrhizinate[33]	PPARα ↑, CPT1/2 ↑	Protect hepatocellular steatosis
Conophylline[34]	PPARα ↑, CPT1/2 ↑	Protect MASLD
Heshouwu[35]	PPARα ↑, CPT1/2 ↑	Protect MASLD

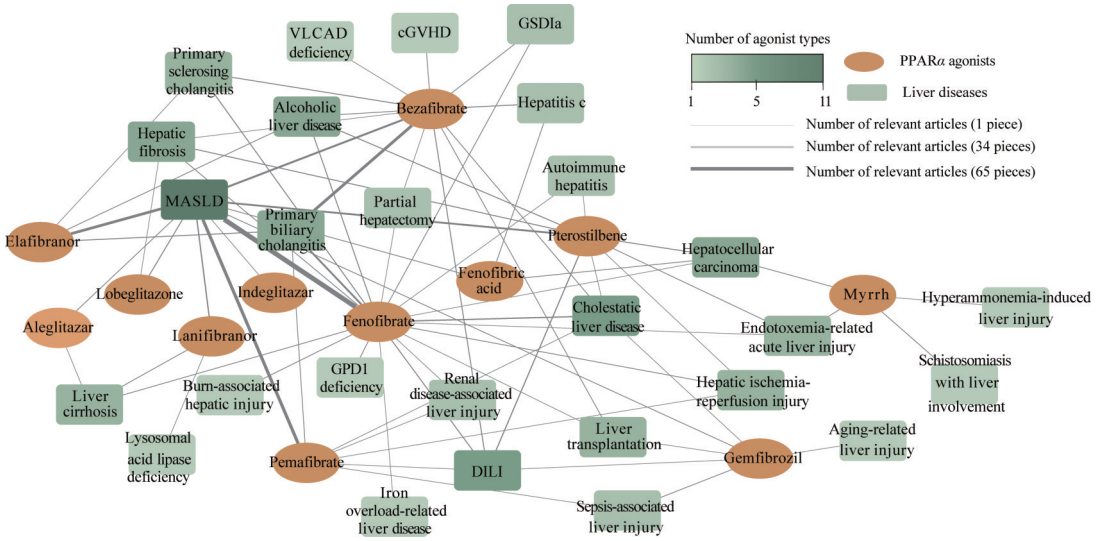


Figure 2 Therapeutic applications of PPAR*α* agonists in liver diseases. Network depicting associations between PPAR*α* agonists (orange nodes) and liver diseases (green nodes). Node color intensity reflects the number of agonist types linked to each disease, and edge width indicates the number of supporting publications per drug-disease pair. cGVHD: Chronic graft-*versus*-host disease; GPD1: Glycerol-3-phosphate dehydrogenase 1; GSDIa: Glycogen storage disease type Ia; MASLD: Metabolic dysfunction-associated steatotic liver disease; VLCAD: Very long-chain acyl-coenzyme A dehydrogenase; DILI: Drug induced liver injury

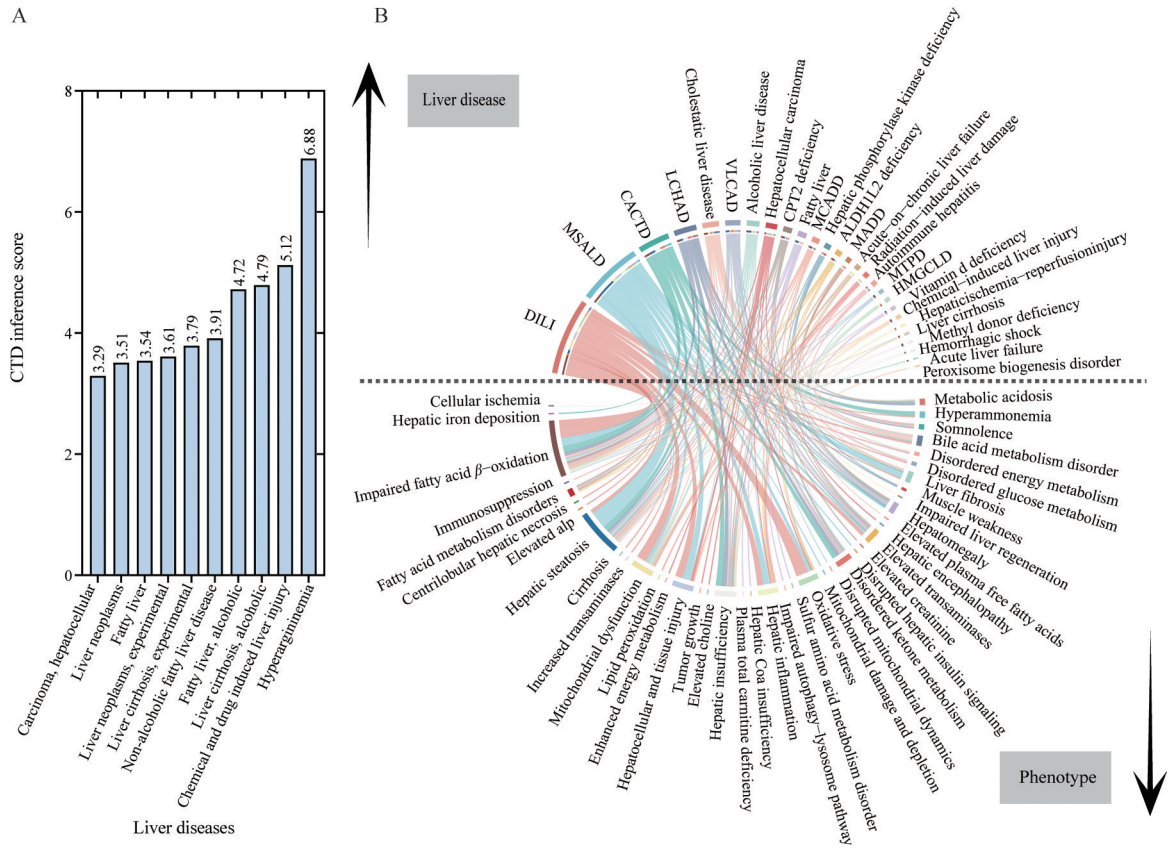


Figure 3 Association between medium- and long-chain acylcarnitines (MCACs/LCACs) and liver diseases. A: Association scores between acylcarnitines and liver diseases calculated from Comparative Toxicogenomics Database (CTD); B: Chord diagram showing liver diseases (top) and pathological phenotypes (bottom) associated with MCACs/LCACs accumulation; arc width represents literature frequency. CACTD: Carnitine-acylcarnitine translocase deficiency; HMGCLD: 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency; LCHAD: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MCADD: Medium-chain acyl-CoA dehydrogenase deficiency; MADD: Multiple acyl-CoA dehydrogenase deficiency; CPT2: Carnitine palmitoyltransferase 2; MTPD: Mitochondrial trifunctional protein deficiency; ALDH1L2: Aldehyde dehydrogenase 1 family member L2

与CON组相比, TP_Cel组血浆ALT、AST与TBA水平均显著升高 ($P < 0.05$, 图4A), 肝组织HE染色显示肝细胞排列紊乱、胞浆疏松伴红细胞漏出 (图4B), 提示肝损伤发生。qPCR结果显示, TP_Cel处理显著下调肝脏 *Ppara*、*Cpt1a*、*Cpt2* 与 *Acox1* 表达 ($P < 0.01$), 提示PPARα信号通路受到抑制 (图4C)。血浆代谢组学分析发现, TP_Cel组C6、C8、C16:1、C18等MCACs/LCACs显著蓄积 ($P < 0.05$, 图4D)。进一步Spearman相关性分析显示, C6、C10、C16:1等MCACs/LCACs与PPARα靶基因表达呈负相关 (相关系数 $\rho = -0.524 \sim -0.970$), 与肝损伤标志物呈正相关 ($\rho = 0.619 \sim 0.976$), 其中大多数相关性达到统计学显著水平 ($P < 0.05$) (图4E)。同时, 与WT-129小鼠相比, *Ppara*$^{-/-}$小鼠血浆中C13-OH、C16:1和C20等LCACs水平亦显著升高 ($P < 0.05$, 图

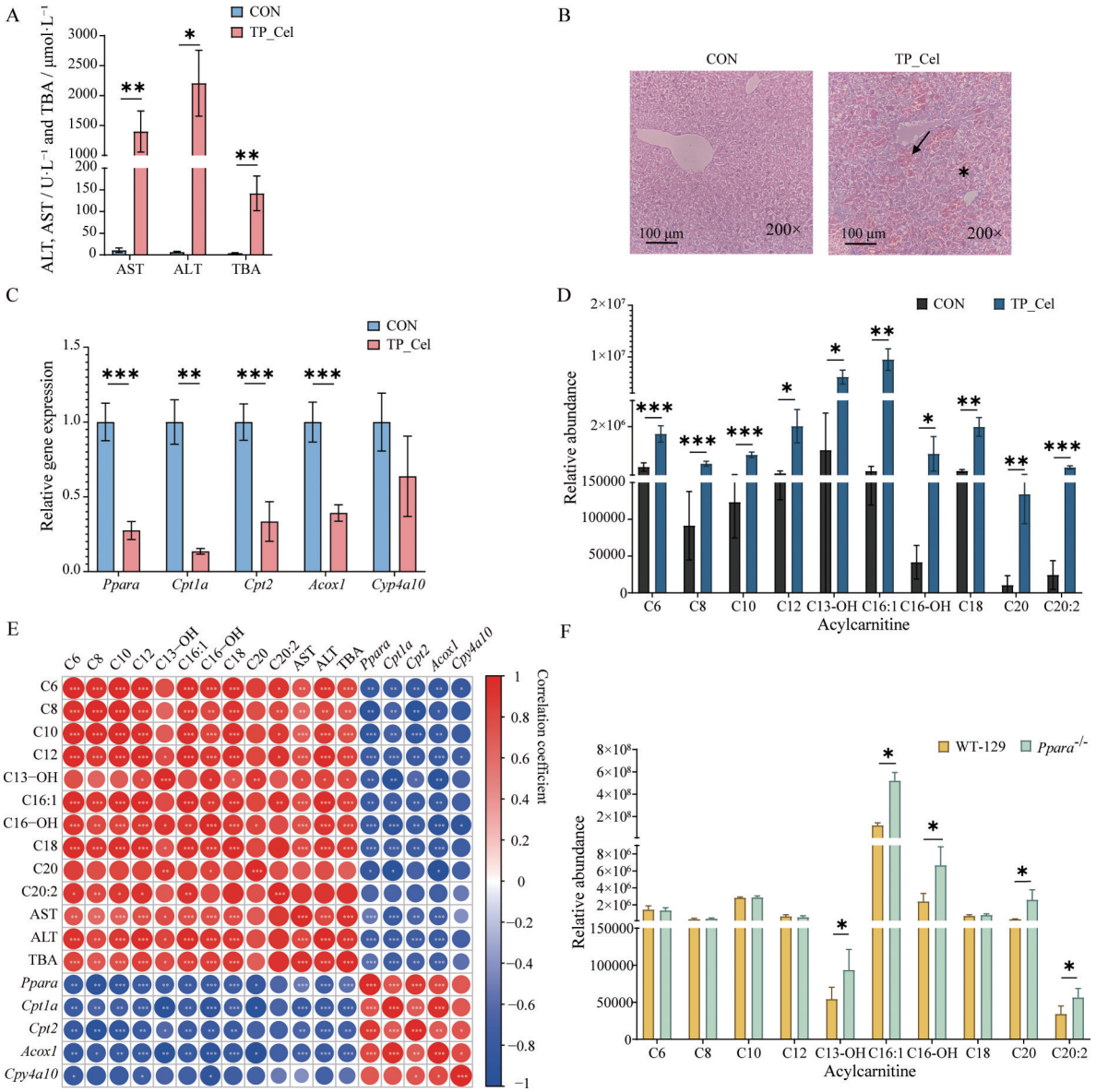


Figure 4 PPARα signaling disruption leads to MCACs/LCACs accumulation in TP_Cel models (A−E) and *Ppara*$^{-/-}$ models (F). A: Plasma ALT, AST, and TBA were significantly elevated, indicating liver injury; B: Representative hematoxylin and eosin (HE) stained liver sections. CON (left): Normal architecture. TP_Cel (right): Hepatocyte disarrangement, cytoplasmic loosening, vacuolar degeneration (*), erythrocyte extravasation (arrows). Scale bar: 100 μm; C: Hepatic mRNA expression of PPARα signaling genes (*Ppara*, *Cpt1a*, *Cpt2*, *Acox1*, *Cyp4a10*) significantly downregulated; D: Plasma MCACs/LCACs abundances were significantly elevated in TP_Cel group compared to CON group; E: Spearman correlation heatmap showed negative correlations between plasma MCACs/LCACs abundances and hepatic PPARα-target gene expression, and positive correlations with liver injury markers; F: Plasma MCACs/LCACs abundances were significantly elevated in *Ppara*$^{-/-}$ group compared to WT-129 group. TP_Cel: Triptolide and celastrol; ALT: Alanine aminotransferase; AST: Aspartate transaminase; TBA: Total bile acid. $n = 4$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* CON group or WT-129 group

4F), 进一步支持了PPARα在MCACs/LCACs稳态维持中的关键调控作用。综上所述, PPARα-CPT1/2-MCACs/LCACs通路在肝损伤进展中具有重要调控与干预潜力。

4 PPARα类保肝中药的筛选

鉴于上述机制通路作用, 本研究系统挖掘作用于该通路的保肝中药资源, 最终筛选出68种HpHMs (图1)。关联网络进一步呈现保肝复方-HpHMs关系 (图5A; 圆形−中药, 菱形−保肝复方, 菱形颜色−复方功效, 外层-PPARα靶向中药, 中层−候选HpHMs, 内层-HpHMs), 共87个保肝复方含有HpHMs。为评估中药的潜在功效, 研究者将复方按功能归类, 结果发现这些复方以“化湿” (23/87 = 26.44%) 和“理气” (20/87 = 22.99%) 类占比最高。节点的网络分析发现 (图5B), 甘草与当归高频地出现在多种保肝复方中。此外, 研究者还探究了HpHMs在肝保护作用领域上的关注度, 其中黄芪、水飞蓟与姜黄在现代肝病研究中引用频率较高, 表明有着一定的研究基础 (图5C)。

5 PPARα类保肝成分的筛选及结构功能分析

为明确HpHMs的活性物质基础, 从68种HpHMs中进一步筛选出29种HpHIs (图1)。HpHIs-HpHMs网络分析显示 (图5E; 绿色-HpHIs, 蓝色-HpHMs, 绿色大小−分布占比), HpHIs分布呈多样性特征, 如槲皮素广泛分布于黄芪、甘草、水飞蓟等28种HpHMs中, 而水飞蓟宾只出现在水飞蓟中。文献计量分析表明, 槲皮素为肝病研究高频成分, 水飞蓟宾、小檗碱及甜菜碱次之 (图5D)。为增加对成分的了解, 进一步统计29种HpHIs的化学类型 (表3)。结果显示, 这些成分以黄酮类为主, 其次为萜类、木脂素类、酚酸衍生物、生物碱类及类固醇类, 另有少量香豆素类和小肽类。

为评估29种HpHIs与PPARα (PDB: 6KAZ) 的结合能力, 进行分子对接模拟 (表3)。除熊果酸外, 其余HpHIs均可嵌合于6KAZ的结合口袋, 结合自由能均低于−10 kcal·mol^{-1}, 显示较强结合能力。从分子相互作用分析, 28种HpHIs可通过范德华力结合, 79.31% (23/29) 形成氢键, 17.24% (5/29) 存在芳香堆叠。结合能最强的5个HpHIs构象显示 (图6), 部分关键残基在结合中可能具有重要作用。如SER280和VAL332分别为6KAZ与不同配体 (槲皮素/小檗碱、槲皮素/芝麻素) 的共同氢键作用位点, 而TYR314参与6KAZ与芝麻素的氢键形成。另外, 橙皮油素虽未形成氢键, 但其通过范德华力、疏水嵌合及芳香堆叠与PPARα稳定结

Table 3 Summary of molecular docking results for HpHIs targeting PPARα (PBD: 6KAZ). H-bond: Hydrogen bond; vdW: van der Waals

HpHIs	Affinity/kJ·mol^{-1}	Interaction	Chemical class (NP Classifier)
Pemafibrate	−40.815	H-bond, vdW, hydrophobic, aromatic stacking	/
Berberine	−38.012	H-bond, vdW, hydrophobic, aromatic stacking	Tyrosine alkaloids
Auraptene	−36.451	vdW, hydrophobic, aromatic stacking	Coumarins
Sesamin	−36.028	H-bond, vdW, hydrophobic, aromatic stacking	Lignans
γ-Tocopherol	−35.572	H-bond, vdW, hydrophobic	Meroterpenoids
Quercetin	−35.192	vdW, H-bond, hydrophobic	Flavonoids
Naringenin	−34.439	vdW, hydrophobic	Flavonoids
Wogonin	−34.158	vdW, hydrophobic	Flavonoids
Apigenin	−34.054	H-bond, vdW, hydrophobic	Flavonoids
Nobiletin	−33.648	H-bond, vdW, hydrophobic	Flavonoids
Nuciferine	−33.439	H-bond, vdW, hydrophobic	Tyrosine alkaloids
Cyanidin	−33.254	H-bond, vdW, hydrophobic	Flavonoids
Eupatilin	−32.924	H-bond, vdW, hydrophobic	Flavonoids
Genistein	−32.037	H-bond, vdW, hydrophobic	Isoflavonoids
Daidzein	−31.974	H-bond, vdW, hydrophobic	Isoflavonoids
Ergosterol	−31.824	vdW, hydrophobic	Steroids
Silibinin	−29.974	H-bond, vdW, hydrophobic, aromatic stacking	Lignans, flavonoids
β-Ecdysone	−28.221	H-bond, vdW, hydrophobic	Steroids
Hesperidin	−28.070	H-bond, vdW, hydrophobic	Flavonoids
Farnesol	−27.573	H-bond, vdW, hydrophobic	Sesquiterpenoids, monoterpenoids
Abscisic acid	−26.259	H-bond, vdW, hydrophobic	Apocarotenoids
Icariin	−26.204	H-bond, vdW, hydrophobic	Flavonoids
Caffeic acid	−25.614	H-bond, vdW, hydrophobic	Phenylpropanoids
Cinnamic acid	−24.246	H-bond, vdW, hydrophobic	Phenylpropanoids
Loganic acid	−23.782	H-bond, vdW, hydrophobic	Monoterpenoids
Gallic acid	−23.326	H-bond, vdW, hydrophobic, aromatic stacking	Phenolic acids
Caryophyllene	−20.188	vdW, hydrophobic	Sesquiterpenoids
Betaine	−14.439	H-bond, vdW, electrostatic	Small peptides
Gomisin a	−13.531	H-bond, vdW, hydrophobic	Lignans
Ursolic acid	/	/	Triterpenoids

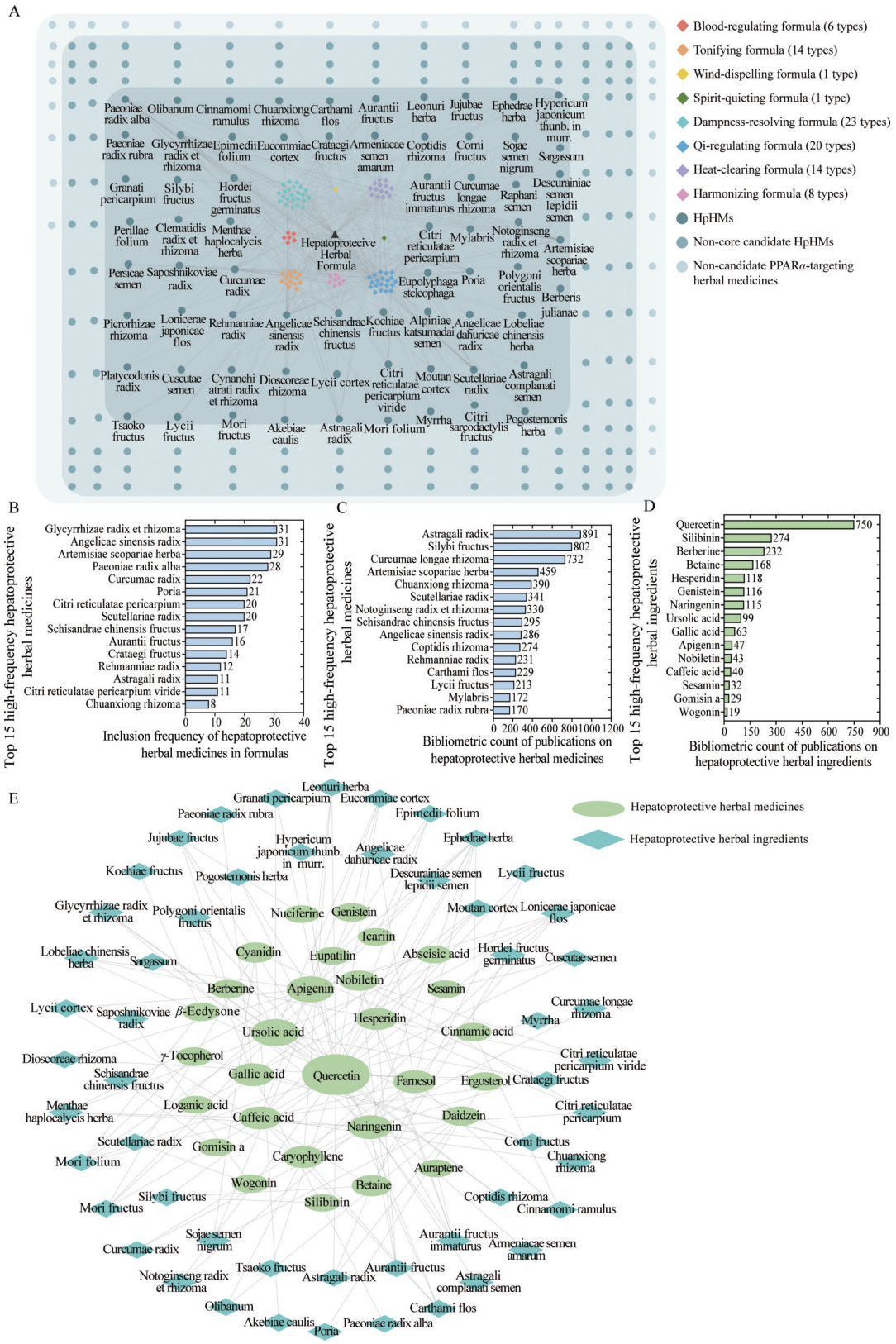


Figure 5 Screening of HpHMs and HpIMs. A: Network diagram of associations among hepatoprotective formulas (diamond nodes), therapeutic functions, and PPARα-targeting herbal medicines (circular nodes). Hierarchical layers: outer (PPARα-targeting herbal medicines), middle (candidate HpHMs), inner (HpHMs); B: Frequency distribution of top 15 HpHMs across NMPA-approved hepatoprotective formulas; C: Bibliometric count of publications for the top 15 HpHMs; D: Bibliometric count of publications for the top 15 HpHIs; E: Network diagram of associations among HpIMs (green nodes) and HpHMs (blue nodes). Node size of HpHIs indicates the number of associated HpHMs

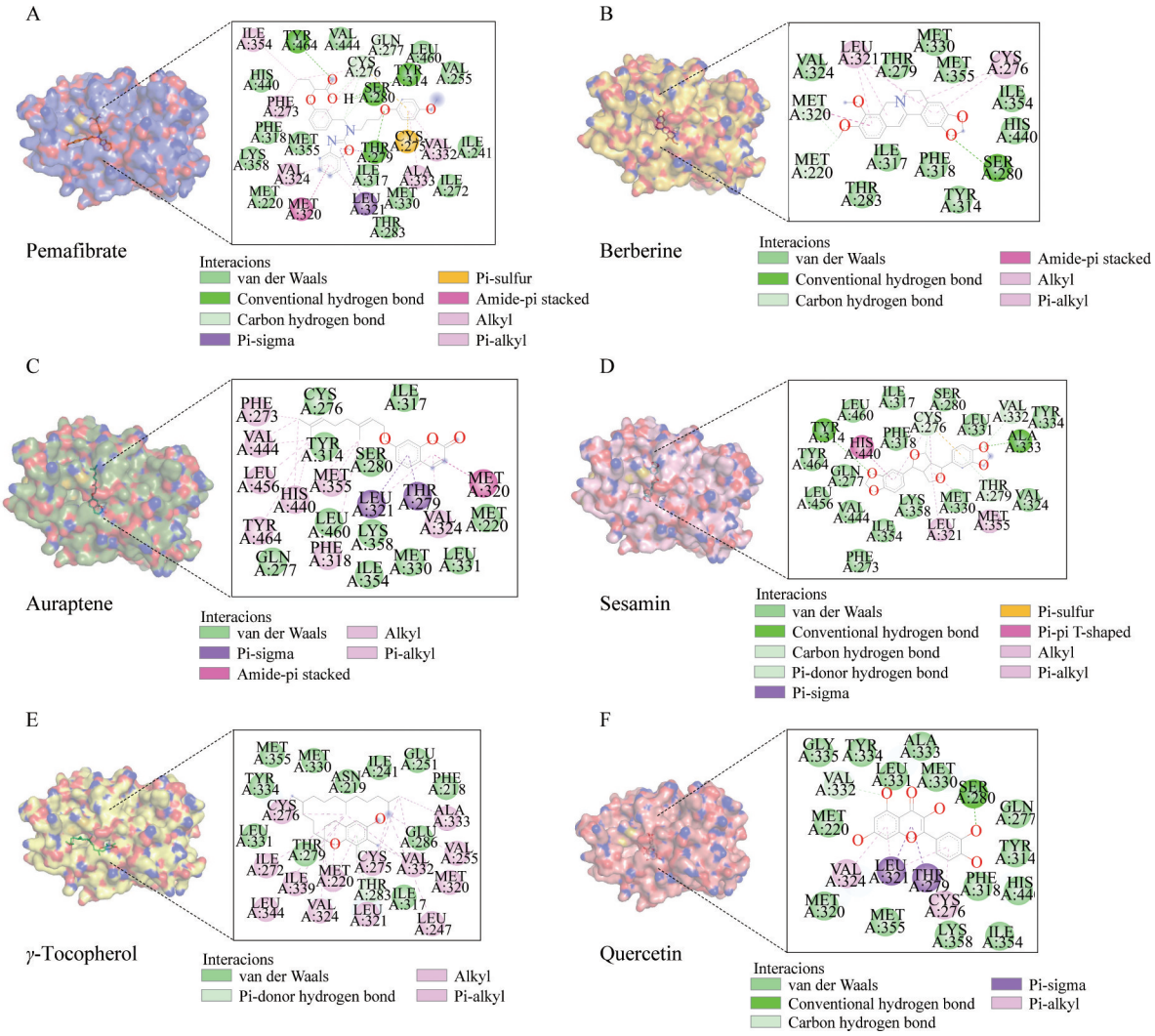


Figure 6 Molecular docking conformations (surface and 2D interaction diagrams) of the top five HpHIs and PPARα agonist pemafibrate with PPARα protein (PBD: 6KAZ)

合, 表现出一定的结构结合特异性。上述结构差异为其与PPARα蛋白不同结合模式的形成提供了重要的结构基础。

讨论

已有研究表明, 临床使用的PPARα激动剂如苯扎贝特可改善MCACs/LCACs代谢紊乱[36], 但中药干预该通路的研究仍较匮乏。本研究结合数据库检索与文献归纳, 初步建立了中药-PPARα-MCACs/LCACs的调控轴, 并在TP_Cel联合诱导肝损伤模型及*Ppara*$^{-/-}$小鼠中进行了验证。结果显示, TP与Cel联用显著抑制*Ppara*表达, 伴随*Cpt1a*、*Cpt2*下调及MCACs/LCACs蓄积; 而*Ppara*$^{-/-}$小鼠亦表现出LCACs水平显著升高, 进一步支持该通路的关键作用。

基于上述PPARα通路在肝损伤中的关键作用, 研究者系统筛选出68种HpHMs与29种HpHIs作为潜在作用于PPARα通路的中药资源。其中, 含有HpHMs的复方主要归入“化湿”与“理气”类, 提示相关中医药理论与现代代谢机制可能存在交叉契合之处。代表性中药和成分如黄芪、水飞蓟、水飞蓟宾与槲皮素等, 均被证实可激活PPARα通路, 改善脂质代谢, 缓解肝病表型[29,37-39], 其中水飞蓟宾和槲皮素亦可缓解TP诱导的肝毒性[40,41]。本研究进一步通过分子对接揭示了多数HpHIs可稳定结合PPARα配体结合口袋, 形成较强的氢键、疏水作用与芳香相互作用, 体现出一定的结构特异性与结合一致性。

需要指出的是, 熊果酸虽未有效嵌合, 但研究表明其可作为PPARα激动剂, 可能通过诱导内源性配体间接激活PPARα[42]。此外, 数据库覆盖范围、结构柔性与电子参数未被全面考虑仍为本研究局限。中药多成分、多靶点特性使其作用受剂量、配伍及个体差异等多因素影响, 仍需进一步通过系统药理、动物实验与临床

研究明确其有效性与安全性。

综上, 本研究系统整合文献、数据库与动物实验数据, 验证了PPARα-MCACs/LCACs通路在脂肪酸氧化障碍相关肝病中的关键作用, 并提出该通路可作为中药干预肝病的重要代谢靶点, 为中药治疗脂质代谢紊乱型肝病提供了新的理论支持与候选药物资源。

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利益冲突: 本研究不存在任何利益冲突。

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