

基于整合文献挖掘和网络药理学探讨黄葵胶囊治疗糖尿病肾病的作用机制

李文鲜¹, 雷 艳¹, 李梦雨¹, 陈 瑞², 邵远洋¹, 刘传鑫^{1, 3*}, 袁瑞娟^{1*}

1. 北京中医药大学 中药学院, 北京 100029

2. 天津中医药大学 中医药研究院, 天津 301617

3. 河南科技大学临床医学院, 河南科技大学第一附属医院内分泌代谢中心 内分泌代谢科, 河南省遗传罕见病医学重点实验室, 国家代谢性疾病临床医学研究中心洛阳分中心, 河南 洛阳 471003

摘 要: 目的 基于整合文献挖掘和网络药理学探究黄葵胶囊治疗糖尿病肾病(DN)的作用机制。方法 通过中国学术期刊全文数据库(CNKI)、万方数据库和Web of Science文献检索平台获取黄蜀葵花化学成分,并借助PubChem数据库与ChemBioDraw软件下载化合物结构;借助Swiss Target Prediction和Stitch数据库预测化学成分相应靶点,并于CTD、DrugBank、GeneCards和TTD数据库中获得DN靶点取交集,得到“成分-疾病”直接共有靶点;利用GeneMANIA获取间接靶点并借助Cytoscape软件构建蛋白质互作网络图;通过基因本体论和京都基因与基因组百科全书数据库(KEGG)对潜在相关靶点进行基因功能富集分析和通路富集分析,并构建黄蜀葵花治疗DN的“成分-直接靶点-间接靶点-通路”网络图。结果 经文献检索得到黄葵胶囊化学成分50个,数据库预测其治疗糖尿病肾病相关靶点182个,KEGG通路富集分析得到187个相关通路,其中6个关键通路可以通过参与调节多个生物过程改善DN疾病的发展。结论 通过文献挖掘与网络药理学初步揭示了黄葵胶囊参与调节机体糖脂代谢、氧化应激、炎症反应、血流动力学紊乱和自噬等生物过程改善DN疾病的发展。

关键词: 网络药理学; 分子机制; 文献挖掘; 糖尿病肾病; 黄葵胶囊

中图分类号: R285.5 文献标志码: A 文章编号: 1674-6376(2021)10-2214-11

DOI: 10.7501/j.issn.1674-6376.2021.10.024

Mechanism of Huangkui Capsule in treatment of diabetic nephropathy based on literature mining and network pharmacology

LI Wenxian¹, LEI Yan¹, LI Mengyu¹, CHEN Rui², SHAO Yuanyang¹, LIU Chuanxin^{1,3}, YUAN Ruijuan¹

1. School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100029, China

2. Institute of traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China

3. Department of Metabolism and Endocrinology, Endocrine and Metabolic Disease Center, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology; Medical Key Laboratory of Hereditary Rare Diseases of Henan; Luoyang Sub-center of National Clinical Research Center for Metabolic Diseases, Luoyang 471003, China

Abstract: Objective To study the mechanism of Huangkui capsule in the treatment of diabetic nephropathy (DN) based on literature mining and network pharmacology. **Methods** The constituents of *A. manihot* were obtained by CNKI, Wanfang database and Web of Science, and the PubChem database and the ChemBioDraw software were used to download the structures of the compounds. Then, the corresponding targets of chemical constituents were predicted in Swiss Target Prediction and Stitch database, and intersected with the DN targets predicted in CTD, DrugBank, GeneCards and TTD databases to obtain the direct targets of "component-disease". The indirect targets were obtained through GeneMANIA, and the protein interaction network map was constructed with the help of Cytoscape software. Finally, Gene ontology and KEGG database were to conduct the Gene function enrichment analysis and pathway enrichment analysis of potential related targets, and the "component-direct target-indirect target-pathway" network diagram of *A. manihot* in the treatment of DN was constructed. **Results** Totally 50 chemical constituents of *A. manihot* were obtained, 182 targets related to the treatment of DN were predicted and 187 pathways were obtained by KEGG

收稿日期: 2021-02-16

第一作者: 李文鲜,女,在读本科生,研究方向为糖尿病肾病机制研究。E-mail: lwx18813158742@163.com

*通信作者: 刘传鑫,男,博士,讲师,研究方向为分析毒理学与临床分子诊断学研究。E-mail: 15222003775@163.com

袁瑞娟,女,博士,副教授,研究生导师,研究方向为中药成分分析与中药质量控制。E-mail: rjyuan@126.com

pathway analysis, of which 6 key pathways can regulate multiple biological processes to improve the development of DN. **Conclusions** Through literature mining and network pharmacology, this study reveals that Huangkui Capsule can participate in regulating biological processes such as glucose and lipid metabolism, oxidative stress, inflammation, hemodynamic disorders and autophagy to improve the development of DN disease, which provides a basis for further study of related mechanisms.

Key words: network pharmacology; molecular mechanism; literature mining; diabetic nephropathy; huangkui capsule

糖尿病是一种由胰岛素分泌或利用缺陷引起的以慢性高血糖为特征的代谢性疾病。糖尿病肾病(diabetic nephropathy, DN)属于糖尿病慢性并发症,是糖尿病患者的主要死因,主要表现为高血压、蛋白尿、肾功能不全等症状。中医将其归于“消渴”“水肿”“关格”等范畴,主要症候表现为阳虚、气虚、血瘀^[1]。在治疗方面,西医以调节饮食结构、控制血糖、血压、减少蛋白尿为主,但服用西药易产生低血糖、低血压、电解质紊乱等不良反应,临床疗效欠佳;中医药治疗DN,以其改善症状明显、不良反应少,可弥补西药不足的优势,在治疗DN的应用中受到较大关注。

黄葵胶囊为黄蜀葵 *Abelmoschus manihot* (L.) Medic. 干燥花冠的提取物制剂,具有清热除湿、消肿解毒等功效。《嘉祐本草》中最早记载黄蜀葵花“利水通淋,消肿解毒,治疗小便淋及诸恶疮脓水”^[2]。目前,国内外学者已从黄蜀葵花中提取出多种化学成分,包括黄酮类、多糖类及长链烃类等,其中,黄酮类化合物为黄葵胶囊主要活性成分。

现有临床研究数据^[3-5]显示,黄葵胶囊治疗DN具有显著效果,主要作用机制为降低炎症因子水平、抑制肾脏组织非酶糖化及氧化、保护肾小管上皮细胞等,从而改善DN患者肾组织损伤。为深入研究黄葵胶囊治疗DN的分子作用机制,本研究通过文献挖掘结合网络药理学技术,构建黄葵胶囊治疗DN的相关靶点蛋白质互作网络图,并通过通路分析获得关键信号通路,从“成分-靶点-通路”角度分析黄葵胶囊治疗DN的分子机制。

1 方法

1.1 黄葵胶囊化学成分数据库的构建

以“黄蜀葵花”“黄葵胶囊”“*Abelmoschus manihot* (L.) Medic.”为关键词,在中国知网(<https://www.cnki.net/>)、万方(<http://www.wanfangdata.com.cn/index.html>)、维普(<http://lib.cqvip.com/>)、Web of Science (<http://apps.webofknowledge.com/>)、PubMed(<https://pubmed.ncbi.nlm.nih.gov/>)数据库中检索黄葵胶囊化学成分,通过 PubChem (<https://pubchem.ncbi.nlm.nih.gov/>)数据库和 ChemBioDraw

Ultra 14.0 软件下载或绘制化学结构并保存为 SDF 格式。

1.2 黄葵胶囊成分靶点的获取

将黄葵胶囊化学成分的 SDF 格式和 SMILES 格式分别导入 Swiss Target Prediction (<http://swisstargetprediction.ch/>)和 Stitch(<http://stitch.embl.de/>)平台,选择“Homo sapiens”,获取黄葵胶囊成分靶点并进行整合筛重。将靶点输入至 Uniprot (<https://www.uniprot.org/>)数据库,选择“Homo sapiens”,进行靶点名称标准化。

1.3 DN靶点的获取

以“diabetic nephropathy”为关键词,借助 CTD (<https://ctdbase.org/>)、DrugBank (<https://www.drugbank.ca/>)、GeneCards (<https://www.genecards.org/>)和 TTD (<http://bidd.nus.edu.sg/group/cjttd/>)靶点信息库获得DN靶点,并进行整合筛重。将靶点上传至 Uniprot(<https://www.uniprot.org/>)数据库,选择“Homo sapiens”,进行靶点名称标准化。

1.4 直接共有靶点的获取

将“1.2”项中得到的黄葵胶囊成分靶点和“1.3”项中得到的DN靶点导入 Draw Venn Diagram(<http://bioinformatics.psb.ugent.be/webtools/Venn/>)中求取靶点交集,得到直接共有靶点。

1.5 间接靶点的获取

将“1.4”项中得到的直接共有靶点上传至 GeneMANIA (<http://genemania.org/>)蛋白互作分析平台,选择“Homo sapiens”,得到间接靶点和直接-间接靶点蛋白互作网络图。

1.6 PPI网络图的构建

将“1.5”项中所得靶点上传至 String (<https://string-db.org/>)分析平台,选择“Homo sapiens”,进行“Multiple Proteins”分析,并下载 TSV 格式文件。将文件导入 Cytoscape 软件,通过“Tools-Network Analyzer-Network Analysis”进行拓扑学分析,以节点颜色和大小反映靶点度(degree值)大小,以边的颜色和粗细反映靶点间关联度强弱,构建靶点 PPI 网络图。根据 degree 值大小,选择排序前6的靶点作为关键靶点。

1.7 基因本体论(GO)生物分析和京都基因与基因组百科全书(KEGG)通路分析

将“1.5”项中所得靶点导入 String 平台的“Multiple proteins”中,选择“Homo sapiens”,进行 GO 生物分析和 KEGG 通路分析,并下载生物过程(biological process, BP)、细胞成分(cellular component, CC)、分子功能(molecular function, MF)和 KEGG Pathways 的 TSV 文件。

借助 GraphPad 软件对 BP、CC、MF 分析进行可视化处理。将信号通路分为“代谢通路”“疾病通路”“细胞过程通路”“信号转导通路”“有机体系统信号通路”,并分别根据 *P* 值选择前 15 条通路,将其导入 Omicshare (<https://www.omicshare.com/>) 平台

进行通路富集分析,得到各类信号通路气泡图。

1.8 “成分-直接靶点-间接靶点-通路”网络图的构建

将黄葵胶囊“化学成分”“直接靶点”“间接靶点”“通路”分为“成分-直接靶点”“直接靶点-间接靶点”“靶点-通路”3 个文件,导入 Cytoscape 中,通过其 Merge 整合功能,构建黄葵胶囊“成分-直接靶点-间接靶点-通路”网络图。

2 结果

2.1 黄葵胶囊化学成分数据库的构建

通过文献挖掘,得到 50 个黄蜀葵花化学成分,下载其 SDF 格式化学结构,构建成成分数据库。具体信息见表 1。

表 1 黄葵胶囊的化学成分
Table 1 Compounds of *A. manihot*

序 号	PubChem CID	化合物	分子式	筛选依据	类别
1	5280343	quercetin	C ₁₅ H ₁₀ O ₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、诱导 CYP1A2 活性 ^[10] 、保护肾小管 ^[11] 、清除超氧阴离子 ^[11]	黄酮
2	133556493	3,5,7-trihydroxy-2-[4-hydroxy-3- [(2R,3S,4R,5R,6S)-3,4,5-trihydroxy- 6-hydroxymethyl]oxan-2-yl] oxyphenyl]chromen-4-one	C ₂₁ H ₂₀ O ₁₂	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
3	5281643	hyperoside	C ₂₁ H ₂₀ O ₁₂	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、保肝作用 ^[12]	黄酮
4	5281672	myricetin	C ₁₅ H ₁₀ O ₈	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
5	950661	(2S)-2-cyclopentyl-N,2- diphenylacetamide	C ₁₉ H ₁₈ O ₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
6	46735347	5-hydroxy-2-(3-hydroxy-4-methoxy- phenyl)-7,8-dimethoxy-chromen-4- one	C ₁₈ H ₁₆ O ₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
7	5280647	gossypetin	C ₁₅ H ₁₀ O ₈	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
8	14353376	5-hydroxy-7,8,4'-trimethoxyflavone	C ₁₈ H ₁₆ O ₆	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
9	5318606	myricetin 3-O-β-D-glucopyranoside	C ₂₁ H ₂₀ O ₁₃	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
10	10371536	quercetin 3-O-robinobioside	C ₂₇ H ₃₀ O ₁₆	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
11	629964	4',5,7,8-tetramethoxyflavone	C ₁₉ H ₁₈ O ₆	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
12	5486615	cannabiscitrin	C ₂₁ H ₂₀ O ₁₃	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
13	5280805	rutin	C ₂₇ H ₃₀ O ₁₆	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、清除超氧阴离子 ^[11]	黄酮
14	5280804	isoquercitrin	C ₂₁ H ₂₀ O ₁₂	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、保护肾小管 ^[13]	黄酮
15	5490334	hibifolin	C ₂₁ H ₁₈ O ₁₄	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、保护肾小管 ^[13]	黄酮
16	44259187	quercetin 3-(6"-acetylglucoside)	C ₂₃ H ₂₂ O ₁₃	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、保护肾小管 ^[13]	黄酮
17	5282160	quercimeritrin	C ₂₁ H ₂₀ O ₁₂	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
18	44259428	myricetin-3-rutinoside	C ₂₇ H ₃₀ O ₁₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
19	44259100	myricetin-3-robinobioside	C ₂₇ H ₃₀ O ₁₆	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
20	44259440	myricetin-3-xyloside	C ₂₀ H ₁₈ O ₁₂	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、保护肾小管 ^[13]	黄酮
21	133613102	gossypetin-8-O-glucuronide	C ₂₁ H ₁₈ O ₁₄	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、保护肾小管 ^[13]	黄酮
22	44259992	hibiscetin-3-glucoside	C ₂₁ H ₂₀ O ₁₄	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮

续表1

序 号	PubChem CID	化合物	分子式	筛选依据	类别
23	14887602	gossypetin-8-glucuronide-3-glucoside	C ₂₇ H ₂₈ O ₁₉	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9] 、保护肾小管 ^[13]	黄酮
24	5320686	tiliroside	C ₃₀ H ₂₆ O ₁₃	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
25	10958572	isopongaflavone	C ₂₁ H ₁₈ O ₄	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
26	5318591	isoliquiritin	C ₂₁ H ₂₂ O ₆	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
27	44229098	quercetin-3,5-O-diglucoside	C ₂₇ H ₃₀ O ₁₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
28	10121947	quercetin-3,7-diglucoside	C ₂₇ H ₃₀ O ₁₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
29	5491941	gossypetin-3,8-O-diglucoside	C ₂₇ H ₃₀ O ₁₈	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
30	44259153	quercetin-3,3'-diglucoside	C ₂₇ H ₃₀ O ₁₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
31	503737	liquiritin	C ₂₁ H ₂₂ O ₆	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
32	5282166	quercetin-3-O-sophoroside	C ₂₇ H ₃₀ O ₁₇	降糖活性 ^[6] 、扩张支气管 ^[7] 、抗炎解热 ^[8] 、镇痛 ^[9]	黄酮
33	5281331	α -spinasterol	C ₂₉ H ₄₈ O	抗炎解热 ^[14]	甾类
34	5280794	stigmasterol	C ₂₉ H ₄₈ O	抗炎、抗氧化、抗肿瘤 ^[15]	甾类
35	222284	β -sitosterol	C ₂₉ H ₅₀ O	降血脂、抗炎、抗肿瘤 ^[16]	甾类
36	5742590	sitogluside	C ₃₅ H ₆₀ O ₆	降糖活性 ^[6]	甾类
37	689043	caffeic acid	C ₉ H ₈ O ₄	降糖活性 ^[6] 、淬灭超氧化物和羟基自由基 ^[17]	有机酸
38	1794427	chlorogenic acid	C ₁₆ H ₁₈ O ₉	淬灭超氧化物和羟基自由基 ^[17]	有机酸
39	370	gallic acid	C ₇ H ₆ O ₅	清除DPPH·和·OH、抑制脂质过氧化 ^[18]	有机酸
40	6474310	isochlorogenic acid A	C ₂₅ H ₂₄ O ₁₂	降糖活性 ^[6]	有机酸
41	6474309	isochlorogenic acid C	C ₂₅ H ₂₄ O ₁₂	降糖活性 ^[6]	有机酸
42	5281780	isochlorogenic acid B	C ₂₅ H ₂₄ O ₁₂	降糖活性 ^[6]	有机酸
43	72	protocatechuic acid	C ₇ H ₆ O ₄	肾保护活性、抗病毒活性、抗糖尿病活性 ^[19]	有机酸
44	12523	phytane	C ₂₀ H ₄₂	含量高、抗氧化、抑菌作用 ^[20]	挥发油
45	12406	pentacosane	C ₂₅ H ₅₂	相对含量较高、抗氧化、抑菌作用 ^[20]	挥发油
46	292286	9-octylheptadecane	C ₂₅ H ₅₂	相对含量较高、抗氧化、抑菌作用 ^[20]	挥发油
47	65575	cedrol	C ₁₅ H ₂₆ O	相对含量较高、抗氧化、抑菌作用 ^[20]	挥发油
48	5319706	methyl linolenate	C ₁₉ H ₃₂ O ₂	相对含量较高、抗氧化、抑菌作用 ^[20]	挥发油
49	23494	tetratetracontane	C ₄₄ H ₉₀	相对含量较高、抗氧化、抑菌作用 ^[20]	挥发油
50	520449	cyclotetracosane	C ₂₄ H ₄₈	相对含量较高、抗氧化、抑菌作用 ^[20]	挥发油

2.2 成分靶点的获取

将“2.1”项中筛选所得50个化学成分的SDF和SMILES格式分别导入Swiss Target Prediction和Stitch平台,分别得到723和19个相关靶点,整理后共得734个靶点。

2.3 DN靶点的获取

在CTD、DrugBank、GeneCards和TTD靶点信息库中检索DN相关靶点,分别得到45、43、829和19个靶点,整理后共888个DN靶点。

2.4 直接共有靶点的获取

将“2.2”项中得到的734个黄葵胶囊成分靶点和“2.3”项中得到的888个DN靶点导入Draw Venn Diagram平台取交集,共得到161个直接共有靶点。

2.5 间接靶点的获取

将“2.4”项中161个直接共有靶点上传至

GeneMANIA蛋白互作平台中进行分析,得到21个间接靶点和直接-间接靶点蛋白互作网络图。结果见图1。

2.6 蛋白质互作网络图的构建

将“2.5”项中潜在作用靶点导入String平台进行分析并下载TSV格式文件,将文件导入Cytoscape软件,进行可视化分析,绘制靶点间蛋白质互作网络图,具体见图2。依据Degree值,取排名前6的靶点作为黄葵胶囊治疗DN的关键靶点,分别为ALB、AKT1、VEGFA、TNF、MAPK3、EGFR。

2.7 GO生物分析和KEGG通路分析

将“2.5”项中潜在作用靶点导入String平台,选择“Homo sapiens”,并下载GO和KEGG通路分析的TSV格式文件。利用GraphPad对GO生物分析进行可视化处理,具体见图3。借助Omicshare平台对

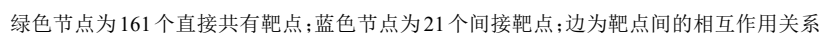


图1 黄葵胶囊治疗DN的靶点

The figure displays a large, intricate network graph representing protein-protein interactions. Nodes are labeled with gene symbols such as PTGS2, STAT3, MAPK8, JUN, VEGFA, AKT1, TNF, MMP9, HRAS, MAPK3, FGF2, EGFR, PPARG, SRC, TP53, KDR, ESR1, ALB, CASP3, IGF1, FN1, MAPK1, MAPK14, APP, IL2, HGF, PDK3S4, MMP2, ICAM1, ACE, HSP90AA1, TLR4, CNDN1, CASP1, ABCG2, SLC22A1, CDK4, ABCB1, CDK6, CDK7, CDK8, CDK9, CDK10, CDK11, CDK12, CDK13, CDK14, CDK15, CDK16, CDK17, CDK18, CDK19, CDK20, CDK21, CDK22, CDK23, CDK24, CDK25, CDK26, CDK27, CDK28, CDK29, CDK30, CDK31, CDK32, CDK33, CDK34, CDK35, CDK36, CDK37, CDK38, CDK39, CDK40, CDK41, CDK42, CDK43, CDK44, CDK45, CDK46, CDK47, CDK48, CDK49, CDK50, CDK51, CDK52, CDK53, CDK54, CDK55, CDK56, CDK57, CDK58, CDK59, CDK60, CDK61, CDK62, CDK63, CDK64, CDK65, CDK66, CDK67, CDK68, CDK69, CDK70, CDK71, CDK72, CDK73, CDK74, CDK75, CDK76, CDK77, CDK78, CDK79, CDK80, CDK81, CDK82, CDK83, CDK84, CDK85, CDK86, CDK87, CDK88, CDK89, CDK90, CDK91, CDK92, CDK93, CDK94, CDK95, CDK96, CDK97, CDK98, CDK99, CDK100, CDK101, CDK102, CDK103, CDK104, CDK105, CDK106, CDK107, CDK108, CDK109, CDK110, CDK111, CDK112, CDK113, CDK114, CDK115, CDK116, CDK117, CDK118, CDK119, CDK120, CDK121, CDK122, CDK123, CDK124, CDK125, CDK126, CDK127, CDK128, CDK129, CDK130, CDK131, CDK132, CDK133, CDK134, CDK135, CDK136, CDK137, CDK138, CDK139, CDK140, CDK141, CDK142, CDK143, CDK144, CDK145, CDK146, CDK147, CDK148, CDK149, CDK150, CDK151, CDK152, CDK153, CDK154, CDK155, CDK156, CDK157, CDK158, CDK159, CDK160, CDK161, CDK162, CDK163, CDK164, CDK165, CDK166, CDK167, CDK168, CDK169, CDK170, CDK171, CDK172, CDK173, CDK174, CDK175, CDK176, CDK177, CDK178, CDK179, CDK180, CDK181, CDK182, CDK183, CDK184, CDK185, CDK186, CDK187, CDK188, CDK189, CDK190, CDK191, CDK192, CDK193, CDK194, CDK195, CDK196, CDK197, CDK198, CDK199, CDK200, CDK201, CDK202, CDK203, CDK204, CDK205, CDK206, CDK207, CDK208, CDK209, CDK210, CDK211, CDK212, CDK213, CDK214, CDK215, CDK216, CDK217, CDK218, CDK219, CDK220, CDK221, CDK222, CDK223, CDK224, CDK225, CDK226, CDK227, CDK228, CDK229, CDK230, CDK231, CDK232, CDK233, CDK234, CDK235, CDK236, CDK237, CDK238, CDK239, CDK240, CDK241, CDK242, CDK243, CDK244, CDK245, CDK246, CDK247, CDK248, CDK249, CDK250, CDK251, CDK252, CDK253, CDK254, CDK255, CDK256, CDK257, CDK258, CDK259, CDK260, CDK261, CDK262, CDK263, CDK264, CDK265, CDK266, CDK267, CDK268, CDK269, CDK270, CDK271, CDK272, CDK273, CDK274, CDK275, CDK276, CDK277, CDK278, CDK279, CDK280, CDK281, CDK282, CDK283, CDK284, CDK285, CDK286, CDK287, CDK288, CDK289, CDK290, CDK291, CDK292, CDK293, CDK294, CDK295, CDK296, CDK297, CDK298, CDK299, CDK300, CDK301, CDK302, CDK303, CDK304, CDK305, CDK306, CDK307, CDK308, CDK309, CDK310, CDK311, CDK312, CDK313, CDK314, CDK315, CDK316, CDK317, CDK318, CDK319, CDK320, CDK321, CDK322, CDK323, CDK324, CDK325, CDK326, CDK327, CDK328, CDK329, CDK330, CDK331, CDK332, CDK333, CDK334, CDK335, CDK336, CDK337, CDK338, CDK339, CDK340, CDK341, CDK342, CDK343, CDK344, CDK345, CDK346, CDK347, CDK348, CDK349, CDK350, CDK351, CDK352, CDK353, CDK354, CDK355, CDK356, CDK357, CDK358, CDK359, CDK360, CDK361, CDK362, CDK363, CDK364, CDK365, CDK366, CDK367, CDK368, CDK369, CDK370, CDK371, CDK372, CDK373, CDK374, CDK375, CDK376, CDK377, CDK378, CDK379, CDK380, CDK381, CDK382, CDK383, CDK384, CDK385, CDK386, CDK387, CDK388, CDK389, CDK390, CDK391, CDK392, CDK393, CDK394, CDK395, CDK396, CDK397, CDK398, CDK399, CDK400, CDK401, CDK402, CDK403, CDK404, CDK405, CDK406, CDK407, CDK408, CDK409, CDK410, CDK411, CDK412, CDK413, CDK414, CDK415, CDK416, CDK417, CDK418, CDK419, CDK420, CDK421, CDK422, CDK423, CDK424, CDK425, CDK426, CDK427, CDK428, CDK429, CDK430, CDK431, CDK432, CDK433, CDK434, CDK435, CDK436, CDK437, CDK438, CDK439, CDK440, CDK441, CDK442, CDK443, CDK444, CDK445, CDK446, CDK447, CDK448, CDK449, CDK450, CDK451, CDK452, CDK453, CDK454, CDK455, CDK456, CDK457, CDK458, CDK459, CDK460, CDK461, CDK462, CDK463, CDK464, CDK465, CDK466, CDK467, CDK468, CDK469, CDK470, CDK471, CDK472, CDK473, CDK474, CDK475, CDK476, CDK477, CDK478, CDK479, CDK480, CDK481, CDK482, CDK483, CDK484, CDK485, CDK486, CDK487, CDK488, CDK489, CDK490, CDK491, CDK492, CDK493, CDK494, CDK495, CDK496, CDK497, CDK498, CDK499, CDK500, CDK501, CDK502, CDK503, CDK504, CDK505, CDK506, CDK507, CDK508, CDK509, CDK510, CDK511, CDK512, CDK513, CDK514, CDK515, CDK516, CDK517, CDK518, CDK519, CDK520, CDK521, CDK522, CDK523, CDK524, CDK525, CDK526, CDK527, CDK528, CDK529, CDK530, CDK531, CDK532, CDK533, CDK534, CDK535, CDK536, CDK537, CDK538, CDK539, CDK540, CDK541, CDK542, CDK543, CDK544, CDK545, CDK546, CDK547, CDK548, CDK549, CDK550, CDK551, CDK552, CDK553, CDK554, CDK555, CDK556, CDK557, CDK558, CDK559, CDK560, CDK561, CDK562, CDK563, CDK564, CDK565, CDK566, CDK567, CDK568, CDK569, CDK570, CDK571, CDK572, CDK573, CDK574, CDK575, CDK576, CDK577, CDK578, CDK579, CDK580, CDK581, CDK582, CDK583, CDK584, CDK585, CDK586, CDK587, CDK588, CDK589, CDK590, CDK591, CDK592, CDK593, CDK594, CDK595, CDK596, CDK597, CDK598, CDK599, CDK600, CDK601, CDK602, CDK603, CDK604, CDK605, CDK606, CDK607, CDK608, CDK609, CDK610, CDK611, CDK612, CDK613, CDK614, CDK615, CDK616, CDK617, CDK618, CDK619, CDK620, CDK621, CDK622, CDK623, CDK624, CDK625, CDK626, CDK627, CDK628, CDK629, CDK630, CDK631, CDK632, CDK633, CDK634, CDK635, CDK636, CDK637, CDK638, CDK639, CDK640, CDK641, CDK642, CDK643, CDK644, CDK645, CDK646, CDK647, CDK648, CDK649, CDK650, CDK651, CDK652, CDK653, CDK654, CDK655, CDK656, CDK657, CDK658, CDK659, CDK660, CDK661, CDK662, CDK663, CDK664, CD

Nodes represent targets and size and color represent score of degree, the nodes become greater, the color changes from green to red, the scores of degree become larger; the edges represent the the degree of interaction between targets, the size and color represent the combine score, the edges become thicker, the color changes from red to green, the combine scores become larger

Fig. 2 PPI network of HKC in treatment of DN

根据 KEGG 通路富集分析结果显示,在得到的

2.8 “成分-直接靶点-间接靶点-通路”网络图的构建

3 讨论

DN是糖尿病的一类重要微血管并发症,也是导致终末期肾脏疾病的主要因素。在西医理论中,代谢紊乱、炎症反应、氧化应激、肾血流动力学改变、自噬等均可作为DN的诱发因素。(1)代谢紊乱:慢性高血糖状态可激活多元醇途径,造成糖基化终产物(advanced glycation end products, AGEs)增多,使AGEs在组织内沉积,改变肾基底膜和血管通透性^[21];糖酵解过程中,肿瘤坏死因子(tumor necrosis factor- α , TNF- α)和转化生长因子 β (transforming growth factor β , TGF- β)的转录得到增强,进一步加重肾脏损伤^[22];DN初期,脂质代谢表现异常,造成脂质沉积,肾小球基底膜通透性提高,肾脏微血管受损。(2)炎症反应:高血糖状态造成炎症因子和细胞黏附因子高表达,内皮细胞通透性提高,蛋白尿

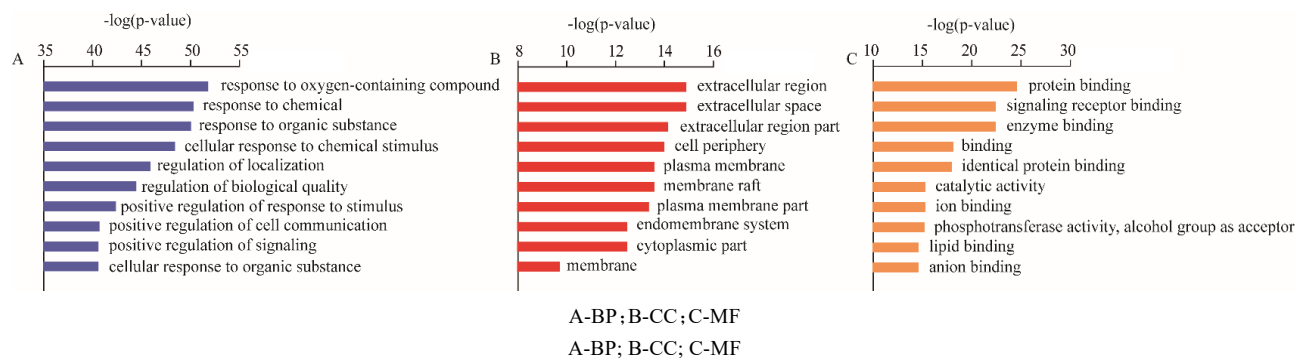
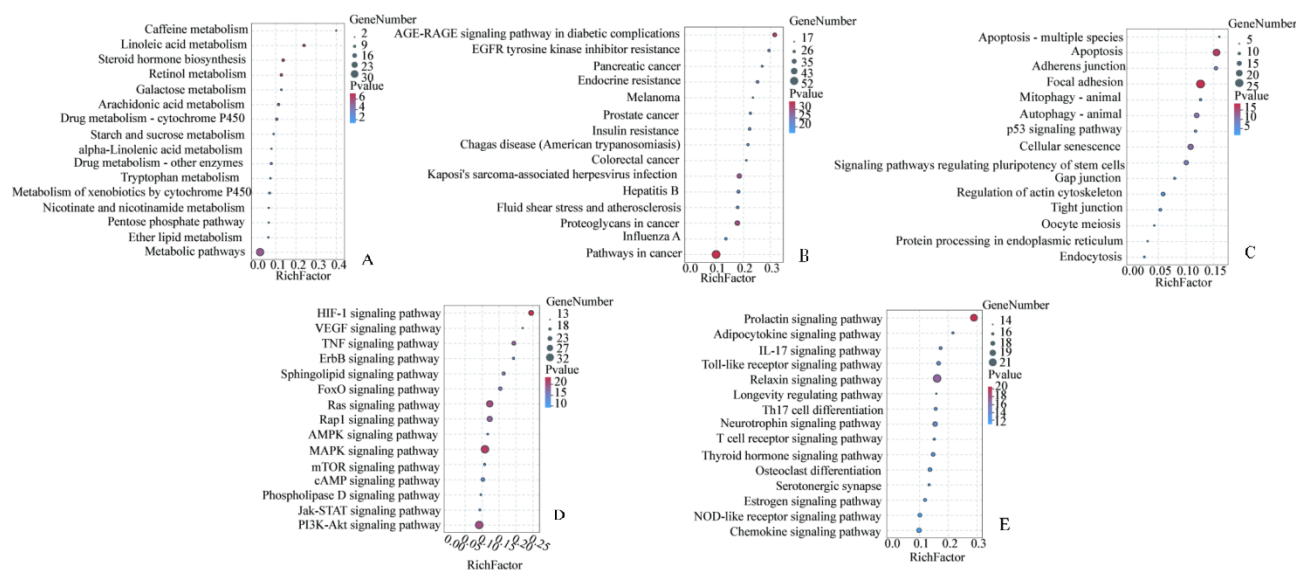


图3 黄葵胶囊潜在作用靶点的GO富集分析

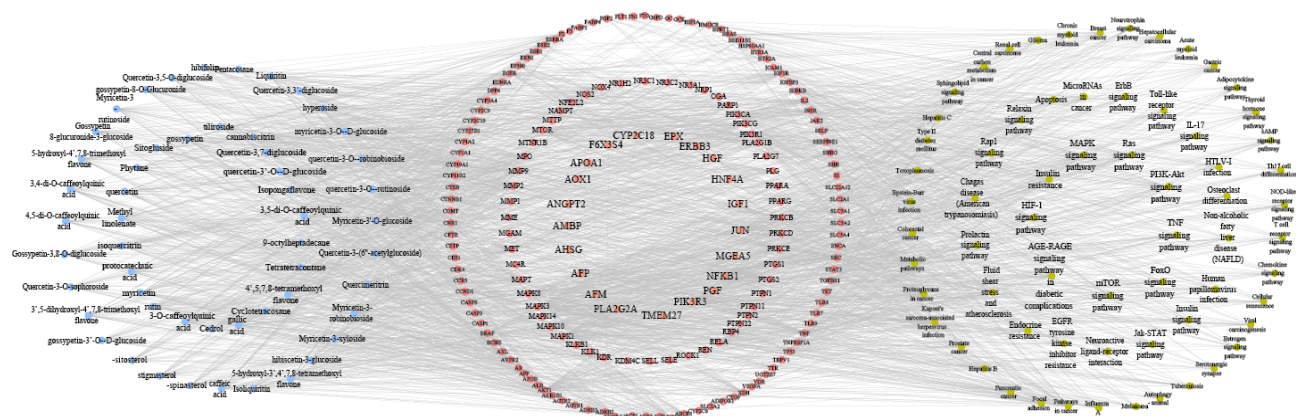
Fig. 3 GO enrichment analysis of HKC potential targets



A-metabolic pathways; B-disease pathways; C-cellular process pathways; D-signal transduction pathways; E-organic system pathways

图4 黄葵胶囊潜在作用靶点的KEGG通路富集分析

Fig. 4 KEGG pathway enrichment analysis of HKC potential targets



蓝色对应化学成分;红色对应直接靶点;橙色对应间接靶点;草绿色对应靶点通路

Blue represents compound; red represents direct target; orange represents indirect target; green represents pathway

图5 黄葵胶囊“成分-靶点-通路”网络

Fig. 5 “Compound-target-pathway” network

水平增加^[21]。(3)氧化应激:活性氧(reactive oxygen species, ROS)可使机体对缺氧的反应性变弱,进而

导致肾小管间质的低氧损伤^[23]。(4)肾血流动力学:肾素-血管紧张素-醛固酮系统(renin-angiotensin-

aldosterone system, RAAS)在高血糖状态下激活,肾小球高压和肾血流动力学改变。(5)自噬:高血糖状态造成细胞自噬活性下降,组织损伤和炎症反应加重^[21]。在中医理论中,DN被认为是“本虚标实”,其发病机制与“肾气阴虚、水湿内停”有关^[24]。在治疗方面,由于西药治疗DN的临床疗效欠佳,发作反复,中成药的治疗效果较为稳定,因此中成药参与临床治疗越来越受关注。

本研究通过文献挖掘获得50个黄葵胶囊化学成分,对成分和疾病靶点进行预测和整合,获得182个潜在靶点,其中ALB、AKT1、VEGFA、TNF、MAPK3和EGFR为关键靶点。对靶点进行GO生物分析和KEGG通路富集分析发现,亚油酸代谢通路(linoleic acid metabolism)、甾体激素生物合成(steroid hormone biosynthesis)代谢通路和HIF-1信号通路(HIF-1 signaling pathway)、MAPK信号通路(MAPK signaling pathway)、肾素血管紧张素信号通路(Ras signaling pathway)、PI3K-Akt信号通路(PI3K-Akt signaling pathway)可以参与调节机体糖脂代谢、氧化应激、炎症反应、血流动力学紊乱和自噬等生物过程,从而改善DN的发展。

3.1 潜在活性成分分析

从化学成分角度分析,金丝桃苷、槲皮素、异槲皮素、杨梅素、槲皮素-3'-O-葡萄糖苷被认为是黄蜀葵花中主要的活性成分^[25-26]。(1)巩春智^[27]研究结果表明金丝桃苷通过降低核因子- κ B的活化来抑制TNF- α 、白细胞介素(IL)-6、IL-1 β 等炎症因子的表达,减轻肾脏炎症反应;张安娜等^[28]研究显示金丝桃苷具有抑制肾小球系膜细胞增殖和凋亡作用,作用机制为降低肾小球系膜细胞活力、抑制高糖刺激TGF- β 1高表达和PI3K-Akt通路下的Bax-Bcl-2蛋白高表达。(2)朱开梅^[29]发现槲皮素能够激活被高糖状态抑制的Smad7信号通路,调节转化因子TGF- β 1的过表达,从而抑制足细胞分泌血管内皮生长因子(vascular endothelial growth factor, VEGF),改善肾纤维化;王兴红等^[30]研究得出槲皮素能够抑制P38-MAPK信号通路和NF- κ B通路,降低炎症因子的释放,减轻炎症反应;宋其蔓等^[31]发现槲皮素在高糖环境下能够激活PI3K-Akt信号通路,具有抗肾小球上皮细胞增殖和氧化应激作用。(3)Kandasamy等^[32]发现杨梅素具有抗氧化和保护肾细胞作用,作用机制为清除氧自由基,调节超氧化物歧化酶(SOD)、过氧化氢酶(CAT)表达,减轻脂质的过氧化;杨梅素还参与糖代谢,通过调节有关糖代谢的

酶和胰岛素信号因子GLUT-2、GLUT-4促进胰岛素分泌,降低血糖浓度^[33];Yang等^[34]研究结果表明杨梅素可以抑制氧化应激和炎症反应,作用机制为调节TGF- β /Smad信号通路,使NF- κ B上调、I κ B α 下调、TGF- β 和VEGF表达下降;Yan等^[35]研究表明杨梅素通过抑制Smad2、MAPK、Akt信号通路可以减轻肾纤维化。

3.2 代谢通路分析

从代谢通路角度分析,亚油酸代谢(Linoleic acid metabolism)和甾体激素生物合成代谢(Steroid hormone biosynthesis)在KEGG分析中富集度较高,其中亚油酸代谢途径主要参与脂质代谢与炎症反应,甾体激素生物合成代谢主要改变肾脏血流动力学和调控机体炎症反应。(1)亚油酸代谢:亚油酸是一类重要的游离脂肪酸,可以促进脂肪细胞增殖分化和细胞内脂肪酸氧化,防止脂质积累过多而在肾脏组织和微血管内沉积^[36]。Christian等^[37]发现亚油酸在内皮细胞炎症反应中具有免疫抗炎作用,作用机制为通过降低TNF- α 、IL-6、细胞间黏附分子-1(ICAM-1)等炎症因子的过表达,调节VEGF,进而作用于细胞增殖和单核细胞迁移而发挥抗炎作用。(2)甾体激素生物合成代谢:甾体激素包括性激素与皮质激素,其中糖皮质激素有效抗炎,盐皮质激素主要参与改善肾血流动力学紊乱。俞文桥^[38]研究发现糖皮质激素的抗炎机制为调节NF- κ B/p38-MAPK-TNF- α 信号通路,使TNF- α 和VEGF表达下调^[39],毛细血管通透性得到改善,内皮细胞损伤减轻,从而发挥抗炎作用;陈颖等^[40]发现盐皮质激素醛固酮能够调节EGFR表达,激活MAPK信号通路,促进细胞增殖;刘抗寒^[41]研究发现醛固酮改变肾脏血流动力学的作用机制为通过盐皮质激素受体上调内皮素1(ET-1)的表达,大幅度收缩入球小动脉,减少肾血浆流量。

3.3 信号转导通路分析

从信号转导通路角度分析,缺氧诱导因子信号通路(HIF-1 signaling pathway)、丝裂原活化蛋白激酶信号通路(MAPK signaling pathway)、Ras signaling pathway、PI3K-Akt signaling pathway等信号通路可以调节多个生物过程延缓DN疾病进程。(1)缺氧诱导因子信号通路:缺氧、炎症、氧化应激等因素均可调节HIF- α 的表达^[42]。低氧条件下,HIF- α 累积并与HIF-1 β 结合,生成的大量HIF能够上调VEGF的表达,造成血管通透性增加、系膜活化、炎症反应加重^[43];此外,HIF- α 的缺氧环境高表

达能够直接激发NF- κ B信号通路,释放TNF- α 等炎症因子。研究发现槲皮素通过抑制HIF脯氨酰羟化酶活性阻碍HIF-VEGF信号通路^[44],金丝桃苷通过抑制PI3K-Akt信号通路,影响其下游基因HIF- α 的表达^[45]。因此,可推测槲皮素、金丝桃苷可能通过抑制HIF信号通路,下调VEGF等细胞因子的表达,减轻机体炎症反应。(2)MAPK信号通路:其中p38-MAPK信号通路,与多种生物过程联系紧密,高血糖、氧化应激、炎症因子及血流动力学紊乱等因素都能够激活p38-MAPK信号通路。当受到细胞因子刺激时,MAPK磷酸化,巨噬细胞浸润,TNF- α 、INF- γ 、IL-6、IL-8炎症因子表达上调,引发炎症反应^[46];当高血糖状态或者机体发生氧化应激时,MAPK/NF- κ B信号通路被激活,VEGF表达上调,一方面血管通透性增加,TNF- α 、TGF- β 、EGF因子渗出,在肾组织系膜上沉积,造成肾脏肥大;另一方面,VEGF上调ET-1的表达,改变肾脏微血管动力学,增加蛋白质通透性,使肾小球高过滤,肾小球肥大^[47]。(3)Ras信号通路:Ras信号通路主要影响肾脏血流动力学,与氧化应激和炎症反应也有一定联系。高血糖状态能够激活RAS系统,使AngII含量增高,一方面激活受体AT1R,刺激VEGF的分泌和表达,提高血管通透性,影响肾脏血流动力学^[48];另一方面AngII可以增加活性氧含量,诱导机体氧化应激,也可以上调TGF- β 1的表达,使细胞外基质合成增多,促进巨噬细胞浸润,诱导炎症反应^[49]。(4)PI3K-Akt信号通路:PI3K被激活后,Akt得到进一步活化。在糖代谢方面,PI3K-Akt信号通路是胰岛素调控血糖平衡的重要信号通路,活化后的Akt一方面磷酸化糖原合成酶激酶-3 β (GSK-3 β),参与糖原的合成,另一方面调控GLUT-4改变骨骼肌细胞摄取利用葡萄糖的速率,调节糖代谢平衡^[50];在调控凋亡方面,Akt阻断下游凋亡因子Bad与Bcl-2/Bcl-xl的结合,抑制细胞凋亡^[51];在细胞自噬方面,Akt进一步活化下游基因mTOR,抑制自噬相关基因Atg1和Atg13复合物的形成,增强核糖体与内质网的粘连性,减轻细胞自噬^[52]。

综上所述,黄蜀葵花主要化学成分金丝桃苷、槲皮素、杨梅素通过调节MAPK、PI3K-Akt、NF- κ B等信号通路作用于VEGF、TNF、EGFR、TGF- β 、HIF- α 靶点,参与调节氧化应激、炎症反应、细胞凋亡以及改变肾血流动力学等生物过程,发挥延缓DN疾病发展的作用,为后续相关机制的深入研究提供一定基础。

4 不足与局限性

由于本研究基于网络平台探究黄葵胶囊治疗糖尿病肾病的分子机制,仍具有不足和局限性:(1)本文主要纳入的是以往研究和成分数据库中收录的黄蜀葵花化学成分,而黄葵胶囊为黄蜀葵花提取物,因此本文的成分研究具有一定局限性;且成分和疾病靶点是由生物信息平台 and 靶点数据库综合整理而来,会影响靶点信息准确性与完整性;(2)未进行分子对接,靶点与成分的吻合度有待进一步检验;(3)化学成分进入机体内需进行代谢过程,活性成分可能改变,本研究所纳入文献中的成分均未进行血清药代实验,活性成分有待通过实验进行验证。

利益冲突 所有作者均声明不存在利益冲突

参考文献

- [1] 杜晓妹,王琳. 中医药治疗糖尿病肾病的研究进展[J]. 现代中西医结合杂志, 2020, 29(23): 2615-2619.
Du X M, Wang L. Study progression in the treatment of diabetic nephropathy with traditional Chinese medicine [J]. Mod J Integr Tradit Chin West Med, 2020, 29(23): 2615-2619.
- [2] 于敏,史耀勋,田谧,等. 黄葵胶囊治疗糖尿病肾病机制探讨[J]. 吉林中医药, 2012, 32(8): 829-831.
Yu M, Shi Y X, Tian M, et al. Mechanism of Huangkui Capsule in treating diabetic nephropathy [J]. J Tradit Chin Med Chin Mater Med Jilin, 2012, 32(8): 829-831.
- [3] 高志田,王刚. 黄葵胶囊对糖尿病肾病患者微炎症状态的观察[J]. 中国中西医结合肾病杂志, 2011, 12(12): 1104-1105.
Gao Z T, Wang G. Mechanism of Huangkui Capsule in treating diabetic nephropathy [J]. Chin J Integr Tradit West Nephrol, 2011, 12(12): 1104-1105.
- [4] 李飞,徐晴晴,赵晓燕,等. 黄葵胶囊联合海昆肾喜治疗慢性肾病的临床研究[J]. 现代药物与临床, 2019, 34(4): 1185-1188.
Li F, Xu Q Q, Zhao X Y, et al. Clinical study on Huangkui Capsules combined with Haikun Shenxi Capsules in treatment of chronic kidney disease [J]. Drugs Clin, 2019, 34(4): 1185-1188.
- [5] 常玲玲,杨淑玲,赵新莲,等. 黄葵胶囊对糖尿病肾病患者肾小管功能的影响[J]. 山东医药, 2009, 49(39): 56-57.
Chang L L, Yang S L, Zhao X L, et al. Effect of Huangkui Capsule on renal tubular function in patients with diabetic nephropathy [J]. Shandong J Tradit Chin Med, 2009, 49(39): 56-57.

- [6] 陈 刚. 黄蜀葵花的化学成分和降糖活性研究 [D]. 北京: 中国人民解放军军事医学科学院, 2006.
Chen G. Studies on the Chemical Constituents and Antihyperglycemic action of *Abelmoschus manihot* L. Medic. [D]. Beijing: Academy of Military Sciences, 2006.
- [7] 王先荣, 王兆全, 李 颖. 黄蜀葵的化学成分研究 [J]. 植物学报: 英文版, 1981(3): 222-227.
Wang X R, Wang Z Q, Li Y. Study on the chemical constituents of *Abelmoschus manihot* [J]. J Integr Plant Biol, 1981(3): 222-227.
- [8] 范 丽, 董六一, 江 勤, 等. 黄蜀葵总黄酮抗炎解热作用 [J]. 安徽医科大学学报, 2003(1): 25-27.
Fan L, Dong L Y, Jiang Q, et al. Anti-inflammatory and antipyretic effects of TFA [J]. Acta Univ Med Anhui, 2003(1): 25-27.
- [9] 范 丽, 董六一, 陈志武, 等. 黄蜀葵总黄酮镇痛作用研究 [J]. 中药药理与临床, 2003(1): 12-14.
Fan L, Dong L Y, Chen Z W, et al. Analgesic effect of total flavone of *Abelmoschus manihot* L. Medic. [J]. Pharmacol Clin Chin Mater Med, 2003(1): 12-14.
- [10] Zhou J Q, Tang Z Q. Effect of quercetin on CYP1A2, CYP2E1, CYP3A2 activities and its inhibitory mechanism studies in rat liver microsomes [J]. J Chin Pharm Sci, 2005, 14(4): 231-236.
- [11] Magnani L, Gaydou E M, de Hubaud J C. Spectrophotometric measurement of antioxidant properties of flavones and flavonols against superoxide anion [J]. Anal Chimica Acta, 2000, 411(1/2): 209-216.
- [12] 温 锐, 谢国勇, 李旭森, 等. 黄蜀葵化学成分与药理活性研究进展 [J]. 中国野生植物资源, 2015, 34(2): 37-44.
Wen R, Xie G Y, Li X S, et al. Advance research on chemical constituents and pharmacological activities of *Abelmoschus manihot* (L.) Medic. [J]. Chin Wild Plant Resour, 2015, 34(2): 37-44.
- [13] Yokozawa T, Dong E B, Kawai Y, et al. Protective effects of some flavonoids on the renal cellular membrane [J]. Exp Toxicol Pathol, 1999, 51(1): 9-14.
- [14] 于凯强, 焦连魁, 任树勇, 等. 中药银柴胡的研究进展 [J]. 中国现代中药, 2015, 17(11): 1223-1229.
Yu K Q, Jiao L K, Ren S Y, et al. Research progress of chinese traditional medicinal *Stellariae Radix* [J]. Mod Chin Med, 2015, 17(11): 1223-1229.
- [15] 周志远, 卢 群, 刘 洋, 等. 豆甾醇的研究及开发进展 [J]. 中国当代医药, 2015, 22(24): 15-17.
Zhou Z Y, Lu Q, Liu Y, et al. Research and development progress of stigmasterol [J]. Chin Mod Med, 2015, 22(24): 15-17.
- [16] 刘威良, 姬 昱, 黄艾祥. β -谷甾醇的研究及开发进展 [J]. 农产品加工, 2019(1): 77-79, 82.
Zhang W L, Ji Y, Huang A X. Research and development progress of β -sitosterol [J]. Proc Agr Prod, 2019(1): 77-79, 82.
- [17] Sova M. Antioxidant and antimicrobial activities of cinnamic acid derivatives [J]. Mini Rev Med Chem, 2012, 12(8): 749-767.
- [18] Lu Z, Nie G, Belton P S, et al. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of Gallic acid derivatives [J]. Neurochem Int, 2006, 48(4): 263-274.
- [19] Kakkar S, Bais S. A review on protocatechuic Acid and its pharmacological potential [J]. ISRN Pharmacol, 2014, 2014: 952943.
- [20] 王 妍. 黄蜀葵及茎叶中黄酮类成分的分析研究 [D]. 北京: 北京中医药大学, 2015.
Wang Y. Studies on analysis of flavonoids of flowers, stems and leaves of *Abelmoschus manihot* (L.) Medic. [D]. Beijing: Beijing University of Chinese Medicine, 2015.
- [21] 刘 菊. 糖尿病肾病的发病机制研究进展 [J]. 黔南民族医专学报, 2015, 28(4): 305-307.
Liu J. Research progress on the pathogenesis of diabetic nephropathy [J]. J Qiannan Med Col Nat, 2015, 28(4): 305-307.
- [22] 孙迎春, 解汝娟. 糖尿病肾病发病机制的研究进展 [J]. 医学综述, 2017, 23(16): 3286-3290.
Sun Y C, Xie R J. Research progress of the pathogenesis of diabetic kidney disease [J]. Med Recapit, 2017, 23(16): 3286-3290.
- [23] 杨 静, 傅继华. 糖尿病肾病病理机制及治疗措施的研究进展 [J]. 医学研究与教育, 2020, 37(1): 1-6.
Yang J, Fu J H. Advances on pathological mechanism and treatment of diabetic nephropathy [J]. Med Res Edu, 2020, 37(1): 1-6.
- [24] 张晓利. 黄葵胶囊在糖尿病肾病中的作用及其机制 [D]. 新乡: 新乡医学院, 2014.
Zhang X L. The Application and Mechanism of Huang Kui Capsule in Diabetic Nephropathy [D]. Xinxiang: Xinxiang Medical University, 2014.
- [25] 池玉梅, 朱华云, 居 玲, 等. 高效液相-四极杆飞行时间串联质谱分析黄蜀葵中黄酮醇类化合物 [J]. 分析化学, 2009, 37(2): 227-231.
Chi Y M, Zhu H Y, Ju L, et al. HPL phase-quadrupole time-of-flight tandem mass spectrometry analysis of flavonols in *Abelmoschus manihot* [J]. Anal Chem, 2009, 37(2): 227-231.
- [26] 李 永, 葛兆宏, 段琼辉, 等. 黄蜀葵中活性成分含量测定 [J]. 亚太传统医药, 2014, 10(22): 16-18.
Li Y, Ge Z H, Duan Q H, et al. Content determination of

- active constituents in *Abelmoschi Corolla* [J]. Asia-Pac Trad Med, 2014, 10(22): 16-18.
- [27] 巩春智. 金丝桃苷对脂多糖所致小鼠急性肾损伤的保护作用及其机制研究 [D]. 济南: 山东大学, 2019.
- Gong C Z. Protective effect of hyperoside on LPS-induced acute kidney injury and the underlying mechanisms [D]. Jinan: Shandong University, 2019.
- [28] 张安娜. 芪术颗粒对糖尿病肾病与肾小球系膜细胞凋亡的影响及机制研究 [D]. 北京: 中国中医科学院, 2015.
- Zhang A N. Effect of Qizhu Granule on diabetic nephropathy and mesangial cell apoptosis and its mechanism [D]. Beijing: China Academy of Chinese Medical Sciences, 2015.
- [29] 朱开梅, 唐丽霞, 赵文鹏, 等. 槲皮素脂质体对糖尿病肾病氧化应激和TGF- β 1/Smad7通路的影响 [J]. 安徽医科大学学报, 2017, 52(3): 319-323.
- Zhu K M, Tang L W, Zhao W P, et al. Effect of quercetin liposomes on oxidative stress and TGF- β 1/Smad7 pathway in kidney of diabetic rats [J]. Acta Univ Med Anhui, 2017, 52(3): 319-323.
- [30] 王兴红, 郑亚萍, 孙曼利, 等. 槲皮素对糖尿病大鼠肾脏p38MAPK/NF- κ B信号通路的影响 [J]. 中药药理与临床, 2016, 32(1): 79-82.
- Wang X H, Zheng Y P, Sun M L, et al. Experimental study on influence of quercetin on kidney p38MAPK/NF- κ B signaling pathway in diabetic rats [J]. Pharmacol Clin Chin Mater Med, 2016, 32(1): 79-82.
- [31] 宋其蔓, 徐新禹, 徐林松. 槲皮素减轻高糖条件下人肾小球内皮细胞损伤的实验研究 [J]. 临床和实验医学杂志, 2020, 19(3): 256-260.
- Song Q M, Xu X Y, Xu L S. Experimental study on quercetin reducing the injury of human glomerular endothelial cells under high glucose condition [J]. J Clin Exp Med, 2020, 19(3): 256-260.
- [32] Kandasamy N, Ashokkumar N. Myricetin modulates streptozotocin-cadmium induced oxidative stress in long term experimental diabetic nephrotoxic rats [J]. J Funct Foods, 2013, 5(3): 1466-1477.
- [33] Kandasamy N, Ashokkumar N. Protective effect of bioflavonoid myricetin enhances carbohydrate metabolic enzymes and insulin signaling molecules in streptozotocin-cadmium induced diabetic nephrotoxic rats [J]. Toxicol Appl Pharmacol, 2014, 279(2): 173-185.
- [34] Yang Z J, Wang H R, Wang Y I, et al. Myricetin attenuated diabetes-associated kidney injuries and dysfunction via regulating nuclear factor (erythroid derived 2)-like 2 and nuclear factor- κ B signaling [J]. Front Pharmacol, 2019, 10: 647.
- [35] Geng Y, Sun Q, Li W, et al. The common dietary flavonoid myricetin attenuates liver fibrosis in carbon tetrachloride treated mice [J]. Mol Nutr Food Res, 2017, 61(4): 1600392.
- [36] 魏佳迪. 基于代谢组学的滇黄精抗脂代谢紊乱作用机制研究 [D]. 昆明: 云南中医学院, 2018.
- Wei J D. Effects and mechanisms of *Polygonatum kingianum* on lipid metabolism disorder [D]. Kunming: Yunnan University of Chinese Medicine, 2018.
- [37] Bork C S, Baker E J, Lundbye-Christensen S, et al. Lowering the linoleic acid to alpha-linoleic acid ratio decreases the production of inflammatory mediators by cultured human endothelial cells [J]. Prostaglandins Leukot Essent Fat Acids, 2019, 141: 1-8.
- [38] 俞文桥. 糖皮质激素对重症急性胰腺炎致肾损伤的临床作用和机制研究 [D]. 杭州: 浙江大学, 2019.
- Yu W Q. Clinical effect and mechanism of glucocorticoid on kidney injury associated with severe acute pancreatitis [D]. Hangzhou: Zhejiang University, 2019.
- [39] 王成玉. 血管内皮生长因子表达与ANCA相关性系统性小血管炎并肾损害关系的研究 [D]. 南宁: 广西医科大学, 2006.
- Wang C Y. Relationship between the expression of Vascular endothelial growth factor and ANCA associated systemic vasculitis with nephrosis [D]. Nanning: Guangxi Medical University, 2006.
- [40] 陈颖, 张爱华, 黄松明, 等. 醛固酮通过线粒体源性氧化应激促进表皮生长因子受体活化及肾小球系膜细胞增殖 [J]. 中华肾脏病杂志, 2010, 26(11): 845-850.
- Chen Y, Zhang A H, Huang S M, et al. Mitochondrial derived reactive oxygen species mediates aldosterone-induced epidermal growth factor receptor activation and mesangial cell proliferation [J]. Chin J Kidney Dis, 2010, 26(11): 845-850.
- [41] 刘抗寒. 醛固酮诱导DOT1/AF9调控ET1表达在糖尿病肾病发病机制中的作用 [D]. 长沙: 中南大学, 2010.
- Liu K H. The role of aldosterone-induced endothelin-1 expression mediated by DOT1/AF9 in diabetic nephropathy [D]. Changsha: Central South University, 2010.
- [42] 杨祖飞, 沈颖. 氧化应激、三七皂苷与糖尿病肾病的研究进展 [J]. 中国医学工程, 2020, 28(1): 28-32.
- Yang Z F, Shen Y. Research progress in oxidative stress, notoginsenoside and diabetic nephropathy [J]. Chin Med Engin, 2020, 28(1): 28-32.
- [43] 张陈文. 有氧运动对糖尿病大鼠炎症及血管内皮mTOR、HIF-1 α 、VEGF表达的影响 [D]. 福州: 福建师范大学, 2017.
- Zhang C W. The effect of inflammation and mTOR、

- HIF-1 α 、VEGF in the vascular endothelium of diabetic rats after aerobic exercise [D]. Fuzhou: Fujian Normal University, 2017.
- [44] Jeon H, Kim H, Choi D, et al. Quercetin activates an angiogenic pathway, hypoxia inducible factor (HIF)-1-vascular endothelial growth factor, by inhibiting HIF-prolyl hydroxylase: a structural analysis of quercetin for inhibiting HIF-prolyl hydroxylase [J]. Mol Pharmacol, 2007, 71(6): 1676-1684.
- [45] 李锦松. 金丝桃苷对低氧时大鼠认知功能损伤的改善作用及其抗氧化应激损伤机制 [D]. 广州: 广东药学院, 2015.
- Li J S. Hyperoside attenuated hypoxia-induced memory impairment and mechanism of resisting to oxidative stress injury [D]. Guangzhou: Guangdong Pharmaceutical University, 2015.
- [46] 吴素琪, 席艺轩, 陈俊, 等. 中药治疗慢性肾小球肾炎所涉信号通路研究进展 [J]. 江西中医药, 2020, 51(9): 74-77.
- Wu S Q, Xi Y X, Chen J, et al. Research progress of signal pathway involved in treatment of chronic glomerulonephritis with traditional chinese medicine [J]. Jiangxi J Tradit Chin Med, 2020, 51(9): 74-77.
- [47] 魏倩萍. P38MAPK在糖尿病肾病中的作用机制研究 [D]. 重庆: 重庆医科大学, 2005.
- Wei Q P. The experimental study on the effect of p38MAPK on diabetic nephropathy [D]. Chongqing: Acta Univ Sci Med Chongqing, 2005.
- [48] 刘晓敏. 双重阻断RAS系统对实验性糖尿病肾病大鼠肾组织Sonic hedgehog信号通路激活的影响 [D]. 桂林: 桂林医学院, 2016.
- Liu X M. Effects of dual blockage of rennin-angiotensin system for activation of Sonic hedgehog pathway on renal in experimental diabetes nephropathy rats [D]. Guilin: J Guilin Med Coll, 2016.
- [49] 刘英莉. 肾脏局部肾素血管紧张素系统在糖尿病肾病发生发展中的作用及机制研究 [D]. 上海: 复旦大学, 2006.
- Liu Y L. The role of renal local renin angiotensin system in the progression of diabetic nephropathy and its involved mechanisms [D]. Shanghai: Fudan University, 2006.
- [50] 吴金梅. 基于PI3K/Akt和APN/AMPK信号通路探讨黄芪葛根配伍对糖尿病大鼠骨骼肌糖脂代谢的影响 [D]. 沈阳: 辽宁中医药大学, 2019.
- Wu J M. Based on PI3K/Akt and APN/AMPK signal pathways to explore the effects of Astragalus and Pueraria on the glucose and lipid metabolism of skeletal muscle in diabetic rats [D]. Shenyang: Liaoning University of Traditional Chinese Medicine, 2019.
- [51] 王颖超, 刘运华, 张丰丰, 等. 糖肾平对糖尿病肾病大鼠足细胞凋亡及PI3K/AKT信号通路的影响 [J]. 世界科学技术-中医药现代化, 2019, 21(9): 1909-1916.
- Wang Y C, Liu Y H, Zhang F F, et al. Effect of tangshenping on apoptosis of podocyte and PI3K-AKT signal pathway in diabetic nephropathy rats [J]. World Sci Technol Mod Tradit Chin Med, 2019, 21(9): 1909-1916.
- [53] 刘丰熙, 李刚, 李秀娟, 等. 苦参碱通过抑制PI3K/Akt/mTOR信号通路诱导食管癌Ec109细胞自噬 [J]. 西安交通大学学报: 医学版, 2020, 41(3): 468-472.
- Liu F X, Li G, Li X J, et al. Matrine induces autophagy in Ec109 cells via inhibiting PI3K/Akt/mTOR signaling pathway [J]. J Xi'an Jiaotong Univ: Med Sci, 2020, 41(3): 468-472.

【责任编辑: 兰新新】