芪蛭降糖片对链脲佐菌素诱导大鼠糖尿病足溃疡的保护作用

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摘要: 观察芪蛭降糖片对糖尿病大鼠足溃疡的保护作用, 探索其可能的作用机制。用链脲佐菌素诱导大鼠糖尿病模型, 模型建立后采用人体等效日剂量的芪蛭降糖片、盐酸二甲双胍片和格列本脲片每日1次独立治疗3个月, 治疗2个月后足面皮肤切除造足溃疡模型, 观察糖尿病大鼠足溃疡愈合的动态变化, 检测指标包括血糖、血清血管内皮生长因子 (VEGF)、诱导型一氧化氮合酶 (iNOS)、血浆中凝血因子Ⅲ (FⅢ)、凝血4项 (凝血酶时间TT、活化部分凝血酶原时间APTT、凝血酶原时间PT、纤维蛋白原FIB) 和创面愈合情况, 网络药理学分析芪蛭降糖片保护糖尿病及其足溃疡的机制, 免疫组化检测胰腺组织转化生长因子-β (TGF-β) 和核因子κB (NF-κB) 蛋白表达验证部分网络药理学预测的损伤保护机制。所有动物实验均经河南大学实验动物伦理委员会批准 (许可证号为HUSAM 2016-288)。结果显示, 模型大鼠一直保持高血糖状态, 多饮多食多尿现象明显, 体重进行性降低, 血清VEGF和iNOS增高, 血凝度增加, 表现为FⅢ增加, TT、APTT、PT延长, FIB降低, 足创面愈合缓慢。盐酸二甲双胍片和格列本脲片虽对高血糖、多饮多食多尿和消瘦有改善作用, 但对高凝状态和创面愈合改善作用不明显。芪蛭降糖片降糖作用不及盐酸二甲双胍片和格列本脲片起效快, 但改善多饮多食多尿和消瘦与二者相似, 且明显改善高凝状态和创面愈合, 降低血清VEGF和iNOS。网络药理学分析表明, 芪蛭降糖片通过胰岛素抵抗通路降低高血糖, 通过HIF-1通路改善血凝状态, 通过VEGF通路、MAPK通路和NF-κB通路影响组织损伤过程阻止糖尿病足溃疡, 免疫组化显示芪蛭降糖片能够抑制糖尿病大鼠胰腺组织TGF-β和NF-κB高表达, 从而维护胰岛功能。本研究表明, 芪蛭降糖片有保护胰岛损伤辅助治疗糖尿病和改善糖尿病大鼠足溃疡愈合作用, 是应用前景较好的糖尿病足防治药物。

关键词: 芪蛭降糖片; 链脲佐菌素; 糖尿病足; 网络药理学

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The protective effect of Qizhi hypoglycemic tablet on foot ulcer in streptozotocin-induced diabetes in rats

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Abstract: This study aimed to determine the protective effect of Qizhi hypoglycemic tablet (QZHGT) on foot ulcer in diabetic rats and explore its possible mechanism. Diabetes was induced by streptozotocin injection in rats. The rats received QZHGT (780 mg·kg^{-1}), metformin hydrochloride tablet (Metf, 200 mg·kg^{-1}) or glibenclamide tablet (Glib, 1.5 mg·kg^{-1}) alone *via* intragastric administration once a day for three months. Food ulcer was prepared by foot skin excision after drug therapy lasted for two months, and the dynamic changes in food ulcer healing were determined. During the experiment, blood glucose, serum levels of vascular endothelial growth factor (VEGF) and inducible nitric oxide synthase (iNOS), factor Ⅲ (FⅢ) and four coagulation parameters [thrombin time (TT), activated partial thromboplatin time (APTT), prothrombin time (PT), fibrinogen (FIB)] were detected.

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Finally, the protective mechanisms of QZHGT against diabetes and foot ulcer were analyzed by network pharmacology, and immunohistochemistry was used to confirm the expression of transforming growth factor-β (TGF-β) and nuclear factor κB (NF-κB) in pancreatic tissue. All animal procedures were approved by the Animal Experimentation Ethics Committee of Henan University (permission number HUSAM 2016-288). The results showed that the lasting hyperglycemia, polydipsia, polyphagia, polyuria and body weight lost took place in model rats compared to those in normal rats. These model rats also showed an increase in serum VEGF and iNOS, FⅢ, TT, APTT and PT, and a reduction in FIB and wound healing. Metf or Glib significantly improved hyperglycemia, polydipsia, polyphagia, polyuria and emaciation, but failed to ameliorate hypercoagulation and wound healing. QZHGT showed a similar effect on polydipsia, polyphagia, polyuria and emaciation to Metf or Glib, although it was inferior to them in hypoglycemic action. Importantly, QZHGT significantly improved hypercoagulation and wound healing, and attenuated serum VEGF and iNOS. Network pharmacology revealed that QZHGT decreased hyperglycemia through "insulin resistance pathway", improved coagulation status through "HIF-1 signaling pathway", prevented diabetic foot ulcers through "VEGF signaling pathway", "MAPK signaling pathway" and "NF-κB signaling pathway". Immunohistochemistry showed that QZHGT could inhibit the expression of TGF-β and NF-κB in pancreatic tissue to maintain islet function in diabetic rats. In summary, these data suggest that QZHGT can prevent pancreatic injury for adjunctive hypoglycemia and diabetic foot ulcer treatment, and is a better preventive and therapeutic drug for diabetic foot ulcer.

Key words: Qizhi hypoglycemic tablet; streptozotocin; diabetic foot; network pharmacology

糖尿病足是糖尿病慢性并发症之一, 其发病机制复杂, 愈合十分艰难, 主要表现为长期足部溃疡、反复感染和形成坏疽, 导致患者被迫截肢, 严重者甚至危及生命[1,2]。中医脉络学说认为微血管损伤是糖尿病足溃疡发生和长期不愈的主要原因[3]。糖尿病足属于临床难治性溃疡范畴, 目前糖尿病治疗药物胰岛素、双胍类、磺脲类及胰岛素增敏剂等, 虽然降糖作用肯定, 但并不能阻止糖尿病足等并发症[4]。中医药因不良反应少、疗效持久等在糖尿病及其并发症防治方面有独特优势[5], 已受到广大患者的普遍认可。中药中很多具有生肌长皮作用[6], 现代中医提出了“气阴两虚兼挟血瘀证”的糖尿病发病理论, 为糖尿病及其并发症防治指明了方向[7]。芪蛭降糖片是林兰教授针对糖尿病中医病机, 结合自身多年的糖尿病治疗经验, 遵循祛瘀益气养阴法拟定而成的纯中药复方制剂, 由黄芪、水蛭、地黄和黄精组成, 是中国SFDA批准的防治糖尿病血管并发症的药物[8]。本研究通过动物实验探讨芪蛭降糖片是否对糖尿病足溃疡有促进修复作用, 以期为其应用特色提供理论依据。

材料与方法

药品与试剂　芪蛭降糖片 (广东合威制药有限公司)。盐酸二甲双胍片 (中美上海施贵宝制药有限公司)。格列本脲片 (新乡市常乐制药有限责任公司)。链脲佐菌素 (STZ) (北京索莱宝科技有限公司)。多聚甲醛 (天津市科密欧化学试剂有限公司)。切片石蜡 (石家庄翼马化工有限公司)。二甲苯、双氧水 (天津市德恩化学试剂有限公司)。中性树胶 (中国上海懿洋仪器有限公司)。免疫组化试剂盒 (武汉博士德生物工程有限公司)。诱导型一氧化氮合酶 (iNOS)、血管生长因子 (VEGF) 试剂盒购自南京建成生物工程研究所。转化生长因子 (TGF-β)、核因子 κB (NF-κB) 抗体购自于武汉三鹰生物科技有限公司。欧姆龙 HEA-STP30 血糖试纸购自欧姆龙健康医疗有限公司。标准啮齿动物饲料购自河南省医学实验动物中心。

动物　SPF级Wistar大鼠, 雌雄兼用, 150～180 g, 河南省医学实验动物中心提供, 许可证号SCXK (豫) 2015-0002。所有动物实验均经河南大学实验动物伦理委员会批准 (许可证号为HUSAM 2016-288)。

仪器　血糖仪, 欧姆龙HEA232 (欧姆龙健康医疗有限公司); Multiskan™ FC 酶标仪 (Thermo Fisher 公司); LGR16-W 高速冷冻离心机 (北京京立离心机有限公司); PB-10 酸度计 (武汉华科达实验设备有限公司); SP-600UZ 奥林巴斯数码照相机 (上海印象销售有限公司)。

糖尿病模型建立　大鼠适应1周后, 禁食12 h, 尾静脉注射枸橼酸缓冲液 (0.05 mmol·L^{-1}, pH=4.5) 配制的STZ 60 mg·kg^{-1}, 72 h后采尾静脉1滴用血糖试纸测空腹血糖, 以血糖值≥13.0 mmol·L^{-1}者入选实验鼠; 血糖未达标者于第5天再次尾静脉注射STZ 60 mg·kg^{-1}, 第8天空腹血糖值≥13.0 mmol·L^{-1}者入选实验鼠。

分组与治疗　实验第15天, 再次检测造模大鼠血糖, 剔除空腹血糖值<13.0 mmol·L^{-1}者。将糖尿病造模成功大鼠按血糖高低随机分为4组, 每组18只, 雌雄各

半: ① 模型组: 灌胃0.5%羧甲基纤维素钠 (CMC-Na) 溶液10 mL·kg^{-1}体重; ② 芪蛭降糖片组: 灌胃0.5% CMC-Na配制的芪蛭降糖片混悬液剂量为780 mg·kg^{-1}; ③ 盐酸二甲双胍片组: 灌胃0.5% CMC-Na配制的盐酸二甲双胍片混悬液200 mg·kg^{-1}; ④ 格列本脲片组: 灌胃0.5% CMC-Na配制的格列本脲片混悬液1.5 mg·kg^{-1}。另选正常大鼠雌雄各9只作对照。分组当日开始药物治疗, 每日1次, 连续治疗3个月。

治疗评价　每周检测大鼠的体重、饮水量、饮食量和尿量变化, 治疗后第10、40、90天血糖试纸测空腹血糖; 治疗后第60天每组取大鼠雌雄各5只用3%戊巴比妥钠溶液腹腔注射麻醉大鼠, 在足背消毒后切除一3 mm×3 mm的全层皮肤, 制备糖尿病足溃疡模型。计算创伤愈合率 [创伤愈合率=(造模时创伤面积−治疗组第n天创伤面积)/造模时创伤面积×100%]。正常组用同法施行足部手术。每日无菌棉纱布创面消毒, 记录伤口愈合情况。第92天眼眶静脉丛采血, 分离血浆用于检测FIII和凝血4项; 分离血清, 检测血清VEGF和iNOS。取胰腺3.8%多聚甲醛固定、按以前的方法[9]常规梯度酒精脱水, 石蜡包埋, 切4 μm厚组织切片分别贴附于普通玻璃载玻片和免疫组化专用玻璃载玻片后进行H&E和免疫组化染色, 评价胰岛损伤情况。

检测方法　血糖仪试纸法检测血糖, VEGF、iNOS及FIII按试剂盒说明进行试样处理, 酶标仪测各试样吸光度后按标准曲线计算各试样检测指标含量。凝血4项用血凝分析仪及相应试剂检测。

网络药理学分析　利用中药系统药理学分析平台数据库 (TCMSP, http://ibts.hkbu.edu.hk/LSP/tcmsp.php) 搜索芪蛭降糖片中黄芪、黄精和地黄的主要化学成分, 选用OB≥30%和DL≥0.18的化合物作为潜在活性化合物, 水蛭中的化学成分另通过文献检索获得[10]。从PubChem数据库 (https://pubchem.ncbi.nlm.nih.gov/) 中获取具有化学结构 (*.SMILES) 的相关成分, 将相关化学成分的Mol2格式结构文件上传至Swiss Target Prediction (www.swisstargetprediction.cn/) 和Stitch (http://stitch.embl.de/) 进行靶点预测, 预测得到的全部靶点作为备选靶点, 采用UniProt数据库 (http://www.uniprot.org/) 将备选靶点名称规范化为官方名 (Official Symbol) 和Uniprot ID。结合糖尿病足溃疡的疾病特点, 通过GENECARD数据库 (https://www.genecards.org/), 以“diabetes”、“ulcer”、“inflammation”、“injury”和“coagulation”为关键词收集与糖尿病足病相关的靶点与预测到的靶点进行对比, 筛选出与芪蛭降糖片防治糖尿病足溃疡作用明确相关的治疗靶点。利用STRING (string-db.org/) 绘制“治疗靶点PPI”网络, 将其导入Cytoscape, 利用Cytoscape中Network Analyzer功能分析网络中的重要靶点。通过生物学信息注释数据库 (DAVID, https://david.ncifcrf.gov/) 对治疗靶点进行GO和KEGG通路富集分析, 并用FDR错误控制法 (FDR<0.05) 对P值作检验校正, 最终设定阈值$P<0.05$, 筛选出与芪蛭降糖片防治糖尿病足溃疡作用相关的生物过程和信号通路, 进一步通过Omicshare在线将GO和KEGG富集分析结果可视化, 筛选出防治糖尿病足溃疡相关通路的化合物。

统计学方法　实验结果以均值±标准差 ($\bar{x} \pm s$) 表示, Graphpad prism5.0软件进行统计学处理, 组间差异用单因素方差分析, 两组间比较用t检验, $P<0.05$为有显著性差异, $P<0.01$为有极显著性差异。

结果

1　芪蛭降糖片对糖尿病大鼠一般症状的影响

模型大鼠多饮、多食、多尿和消瘦现象明显, 创面愈合缓慢。盐酸二甲双胍片和格列本脲片对多饮多食多尿和消瘦有改善作用, 但对创面愈合影响不明显。芪蛭降糖片阻止多饮多食多尿和消瘦方面与两常用口服降糖药相似, 且能明显促进创面愈合, 结果见表1～3、图1。

2　芪蛭降糖片对糖尿病大鼠血糖和血清VEGF和iNOS的影响

模型大鼠一直保持高血糖状态, 血清VEGF和iNOS增高。盐酸二甲双胍片和格列本脲片对高血糖有降低作用, 对血清VEGF和iNOS无明显影响。芪蛭

Table 1　QZHGT decreases water and food intake in diabetic rats. n = 18, $\bar{x} \pm s$. $^{*}P<0.05$, $^{**}P<0.01$ *vs* DM; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* Cont. Cont: Control group; DM: Diabetes model group; QZHGT: Qizhi hypoglycemic tablet group; Metf: Metformin tablet group; Glib: Glibenclamide tablet group

Group	Water/mL			Food intake/g		
	10 day	40 day	90 day	10 day	40 day	90 day
Cont	32.00 ± 2.87	33.25 ± 5.56	29.44 ± 7.04	15.00 ± 2.83	16.59 ± 3.56	18.52 ± 3.38
DM	$128.65 \pm 9.71^{\#\#}$	$125.27 \pm 10.20^{\#\#}$	$145.2 \pm 10.93^{\#\#}$	$30.65 \pm 3.10^{\#\#}$	$34.59 \pm 3.23^{\#\#}$	$35.64 \pm 8.00^{\#\#}$
QZHGT	$113.35 \pm 7.27^{*\#}$	$109.61 \pm 9.26^{**\#}$	$113.23 \pm 8.65^{**\#\#}$	$25.53 \pm 1.39^{**\#}$	$28.23 \pm 4.26^{**\#}$	$30.31 \pm 5.74^{\#\#}$
Metf	$108.71 \pm 9.29^{**\#}$	$95.58 \pm 8.68^{**\#}$	$99.58 \pm 7.38^{**\#}$	$25.98 \pm 1.92^{*\#}$	$25.55 \pm 3.13^{**\#}$	$27.06 \pm 1.48^{**\#}$
Glib	$122.57 \pm 7.29^{\#\#}$	$127.71 \pm 5.46^{\#\#}$	$142.31 \pm 9.613^{\#\#}$	$29.02 \pm 2.55^{\#\#}$	$29.50 \pm 4.14^{*\#\#}$	$33.34 \pm 6.42^{\#\#}$

Table 2　QZHGT decreases urine output and body weight in diabetic rats. n = 18, $\bar{x} \pm s$. $^{*}P$<0.05, $^{**}P$<0.01 *vs* DM, $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* Cont

Group	Urine output/g			Body weight/g		
	10 day	40 day	90 day	10 day	40 day	90 day
Cont	14.33 ± 4.84	16.80 ± 1.16	15.17 ± 4.20	226.44 ± 8.15	268.00 ± 10.25	301.25 ± 9.87
DM	111.55 ± 5.44$^{\#\#}$	125.41 ± 4.11$^{\#\#}$	124.88 ± 2.67$^{\#\#}$	178.92 ± 8.88$^{\#\#}$	208.84 ± 7.27$^{\#}$	220.70 ± 5.65$^{\#\#}$
QZHGT	98.59 ± 6.22$^{**\#\#}$	96.63 ± 3.70$^{**\#\#}$	80.65 ± 3.96$^{**\#\#}$	192.25 ± 9.16$^{*\#}$	227.11 ± 7.35$^{**\#}$	232.77 ± 3.87$^{**\#}$
Metf	85.56 ± 5.66$^{**\#\#}$	78.97 ± 5.44$^{**\#}$	79.64 ± 5.08$^{**\#}$	197.20 ± 9.72$^{**\#}$	223.73 ± 10.74$^{**\#}$	262.28 ± 3.12$^{**\#}$
Glib	112.5 ± 4.32$^{\#\#}$	111.33 ± 5.03$^{**\#\#}$	121.61 ± 5.19$^{\#\#}$	198.45 ± 5.32$^{**\#}$	222.62 ± 7.72$^{**\#}$	224.25 ± 9.66$^{\#\#}$

Table 3　QZHGT promotes wound healing in diabetic rats. n = 18, $\bar{x} \pm s$. $^{*}P$<0.05, $^{**}P$<0.01 *vs* DM; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* Cont

Group	Wound healing/%			
	1 day	5 day	10 day	15 day
Cont	0	66.67 ± 12.78	87 ± 3	99 ± 2
DM	0	21 ± 9$^{\#}$	47.33 ± 7.33$^{\#\#}$	85 ± 5$^{\#\#}$
QZHGT	0	44.67 ± 8.78$^{*\#}$	80.67 ± 5.78**	98.33 ± 3.33*
Metf	0	45.67 ± 5.78$^{*\#}$	76 ± 6$^{*\#}$	95.33 ± 2.33$^{*\#}$
Glib	0	36.33 ± 8.33$^{\#}$	65 ± 6$^{\#\#}$	93.67 ± 3.78$^{*\#\#}$

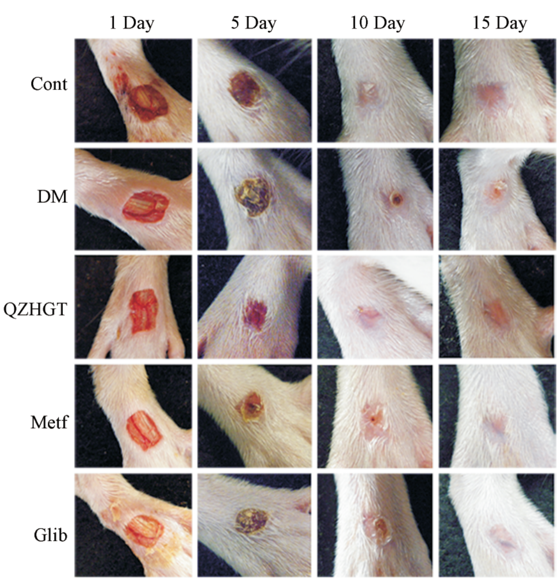

Figure 1　QZHGT promotes wound healing of skin excision in diabetic rats

降糖片降糖作用不及盐酸二甲双胍片和格列本脲片起效快, 但明显改善降低血清VEGF和iNOS, 结果见表4、5。

3　芪蛭降糖片阻止糖尿病大鼠高凝状态

模型大鼠FIII增加, 凝血4项中TT、APTT、PT延长、FIB降低, 表现为高凝状态。盐酸二甲双胍片和格列本脲片对高凝状态影响不明显。芪蛭降糖片明显改善高凝状态, 结果见表6。

Table 4　QZHGT decreases blood glucose in diabetic rats. n = 18, $\bar{x} \pm s$. $^{**}P$<0.01 *vs* DM, $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* Cont

Group	Blood glucose/mmol·L^{-1}		
	10 day	40 day	90 day
Cont	5.41 ± 0.59	5.51 ± 0.56	5.12 ± 0.59
DM	24.04 ± 4.44$^{\#\#}$	23.98 ± 4.98$^{\#\#}$	25.17 ± 5.40$^{\#\#}$
QZHGT	22.44 ± 4.39$^{\#\#}$	18.73 ± 5.87$^{**\#\#}$	16.82 ± 5.60$^{**\#\#}$
Metf	16.96 ± 4.40$^{**\#}$	13.62 ± 4.79$^{**\#}$	10.71 ± 5.73$^{**\#}$
Glib	20.92 ± 5.91$^{\#\#}$	18.01 ± 5.09$^{**\#\#}$	14.83 ± 6.57$^{**\#}$

Table 5　QZHGT decreases serum levels of VEGF and iNOS in diabetic rats. n = 18, $\bar{x} \pm s$. $^{*}P$<0.05 *vs* DM; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* Cont. VEGF: Vascular endothelial growth factor, iNOS: Inducible nitric oxide synthase

Group	iNOS/U·mL^{-1}		VEGF/pg·mL^{-1}	
	40 day	90 day	40 day	90 day
Cont	17.98 ± 1.33	18.51 ± 4.26	65.24 ± 11	67.92 ± 5.16
DM	25.13 ± 14.36$^{\#}$	25.15 ± 8.81$^{\#}$	125.81 ± 33.7$^{\#}$	159.89 ± 62.13$^{\#\#}$
QZHGT	21.23 ± 9.01	20.22 ± 3.48*	118.45 ± 12.16$^{\#}$	105.84 ± 26.64$^{*\#}$
Metf	20.6 ± 7.05	22 ± 3.5$^{\#}$	123.7 ± 40.52$^{\#}$	111.47 ± 28.09$^{*\#}$
Glib	22 ± 1.84$^{\#}$	23.7 ± 1.43$^{\#}$	113.22 ± 30.86$^{\#}$	136.76 ± 57$^{\#\#}$

4　芪蛭降糖片防治糖尿病足溃疡的机制

本实验共检索出129个化学成分, 其中黄芪44个, 黄精22个。地黄26个, 水蛭41个, 其活性成分-靶点网络图见图2。将获得有化学结构成分的靶点信息与GENECARD数据库中糖尿病足溃疡治疗药物靶标比对分析, 归纳总结筛选出163个可能与芪蛭降糖片防治糖尿病足溃疡相关联的潜在作用靶点以及117个相对应的化学成分, 见图3。用PPI网络拓扑分析核心靶的筛选出10个核心靶蛋白, 见图4。GO功能注释预示, 辅助降糖和改善糖尿病足的相关靶点涉及的生物过程 (主要药效分子) 分别是葡萄糖稳态 (西托糖苷、

Table 6　QZHGT improves coagulation parameters in diabetic rats. n = 18, $\bar{x} \pm s$. $^{*}P$<0.05, $^{**}P$<0.01 *vs* DM; $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs* Cont. FIII: Factor III; TT: Thrombin time; APTT: Activated partial thromboplatin time; PT: Prothrombin time; FIB: Fibrinogen

Group	FIII/pg·mL^{-1}	TT/s	APTT/s	PT/s	FIB/g·L^{-1}
Cont	2.79 ± 0.074	21.28 ± 5.43	21.30 ± 0.85	7.80 ± 0.3 6	3.73 ± 0.61
DM	4.24 ± 0.18$^{\#\#}$	32.85 ± 7.01$^{\#\#}$	32.58 ± 3.08$^{\#\#}$	14.66 ± 3.16$^{\#\#}$	1.47 ± 0.64$^{\#\#}$
QZHGT	2.68 ± 0.38*	26.15 ± 4.72$^{\#}$	23.56 ± 2.51**	9.32 ± 0.58$^{**\#}$	2.12 ± 0.42$^{**\#}$
Metf	3.82 ± 0.36$^{\#}$	22.97 ± 4.45	23.05 ± 1.24**	9.14 ± 0.88$^{**\#}$	2.21 ± 0.37$^{**\#}$
Glib	3.64 ± 0.54$^{\#}$	21.4 ± 8.01*	24.58 ± 2.00$^{**\#}$	11.73 ± 3.99$^{\#\#}$	2.99 ± 0.40$^{**\#}$

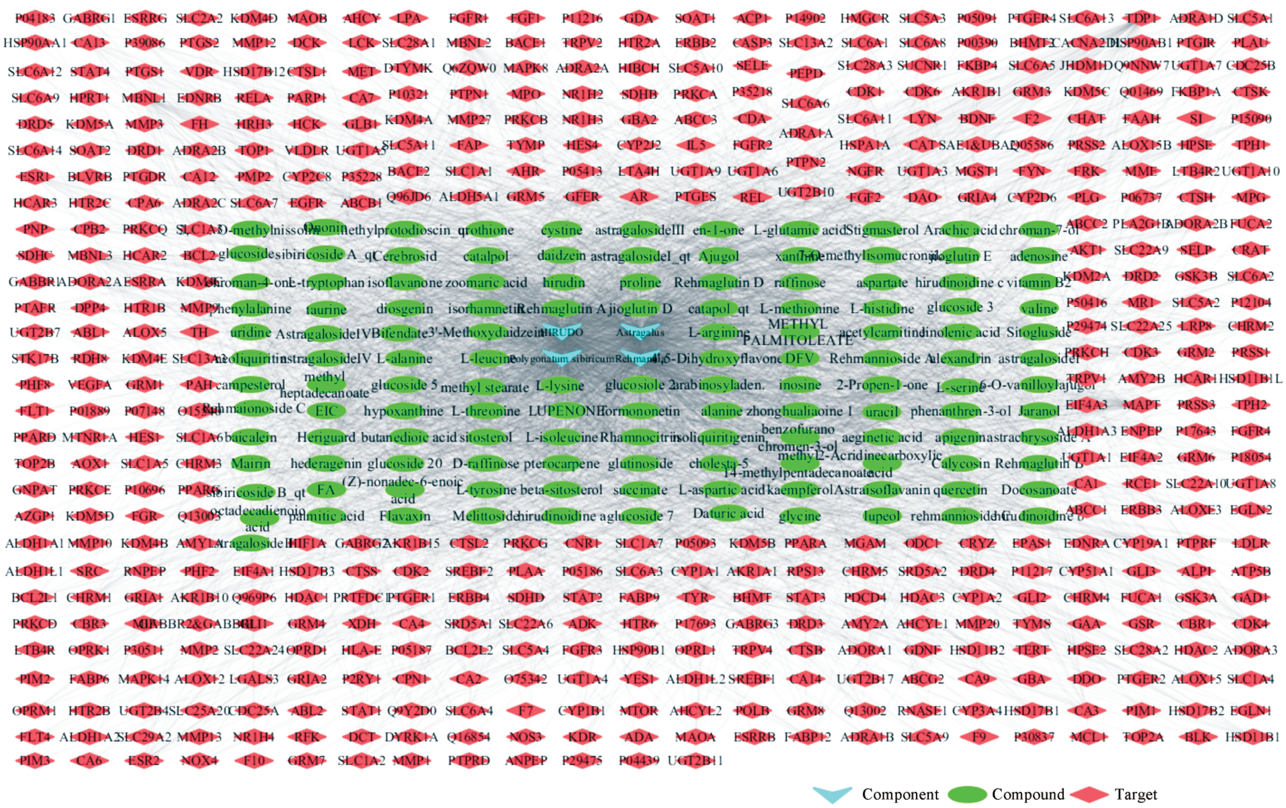

Figure 2　Component-compound-target network of QZHGT

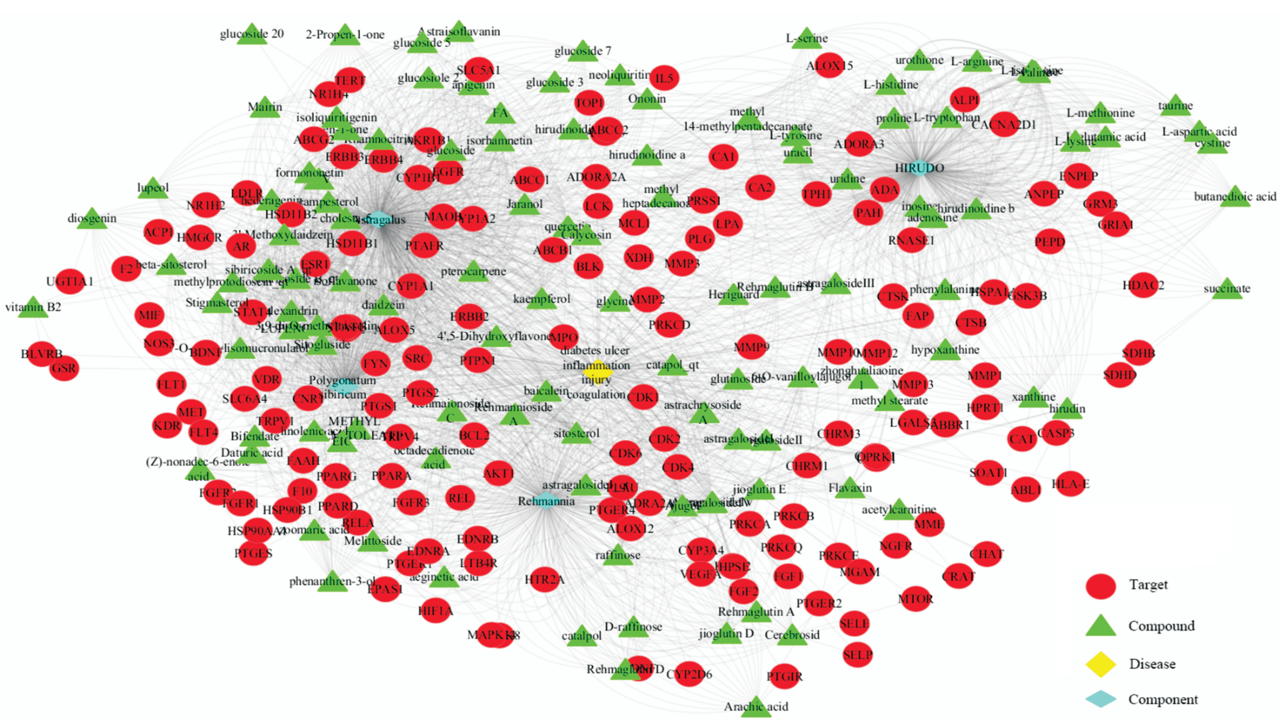

Figure 3　Disease-target-compound-component network of QZHGT-associated active compounds involved in the roles of hypoglycemic and wound healing

大豆苷元和野菰酸等) 和血管生成的正向调节 (黄芩素、大豆苷元和棉子糖等)、胶原分解代谢过程 (黄芩素、槲皮素和毛蕊异黄酮苷等)、炎症反应 (西托糖苷、4',5 二羟基黄酮和野菰酸等)、NF-κB 转录因子活性的正调节 (西托糖苷、地黄苷 C 和谷甾醇等)、伤口愈合 (黄芩素、大豆苷元、野菰酸) 以及细胞黏附的积极调

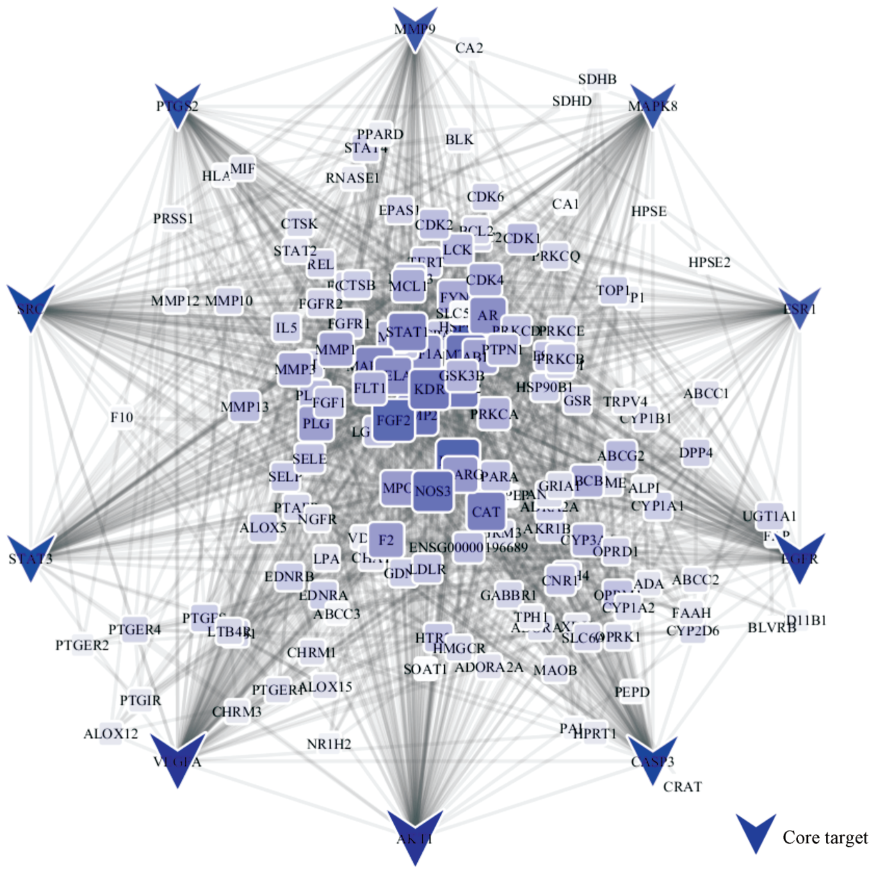

Figure 4 Targets interaction network of QZHGT-associated active compounds involved in the roles of hypoglycemic and wound healing

节 (黄芩素、大豆苷元、槲皮素等), 见图5。KEGG分析结果预示, 芪蛭降糖片中西托糖苷、大豆苷元和黄芩素等通过胰岛素抵抗通路降低高血糖; 西托糖苷、黄芩素和棉子糖等通过HIF-1通路改善血凝状态; 西托糖苷、大豆苷元、黄芩素和棉子糖等通过VEGF通路、MAPK通路和NF-κB通路影响组织损伤过程阻止糖尿病足溃疡, 见图6。

5 芪蛭降糖片对糖尿病大鼠胰岛损伤的保护作用

模型大鼠胰腺组织损伤明显, 表现为细胞水肿变形、胰岛细胞数目减少、弥散, 胰腺组织TGF-β和NF-κB表达增高。盐酸二甲双胍片和格列本脲片对胰腺组织损伤改善作用不明显。芪蛭降糖片明显促进胰腺组织损伤修复, 降低胰腺组织TGF-β和NF-κB表达(图7)。

讨论

糖尿病为慢性代谢障碍性疾病, 其持续高血糖状态能诱发多种并发症[11]。糖尿病并发症累及的脏器功能障碍可导致患者出现相应的病症, 其中糖尿病病足是以毛细血管与神经病变为主要病理改变的糖尿病并发症, 其血管堵塞引起肢体缺血和缺氧诱发的糖尿病足溃疡进一步使病情加重, 严重患者被迫截肢而致残[12-15]。本研究中, 模型大鼠持续保持高血糖状态, 模型成功后即出现明显多饮、多食和多尿现象, 伴随血清VEGF和iNOS升高的血管炎症标志, 创面愈合缓慢。常用口服降糖药盐酸二甲双胍片和格列本脲片降糖作用出现较快 (10天), 但对VEGF和iNOS血管炎症标志及创面愈合均无明显改善。芪蛭降糖片降糖作用出现较慢 (40天), 但能降低VEGF和iNOS血管炎症标志、促进创面愈合, 提示芪蛭降糖片降糖机制与盐酸二甲双胍片和格列本脲片不同。

糖尿病足溃疡难以愈合的原因有多种, 如高血糖持续状态和晚期糖基化终末产物大量蓄积等[16]。长期高血糖可引起周围神经病变及周围血管堵塞, 当病变出现在下肢导致局部皮肤破溃时就形成了糖尿病足病, 创面长期难以愈合。中医认为糖尿病患者气阴两虚导致的气滞血瘀是糖尿病足病发生的关键, 行气活血可以改善创面微循环, 为创伤修复提供营养物质和氧气, 从而促进创面新陈代谢和溃疡愈合。在本研究中, 模型大鼠出现FIII增加, 凝血4项中TT、APTT、PT延长、FIB降低, 表现为高凝状态。常用口服降糖药盐酸二甲双胍片和格列本脲片虽然降糖和减轻多饮、多

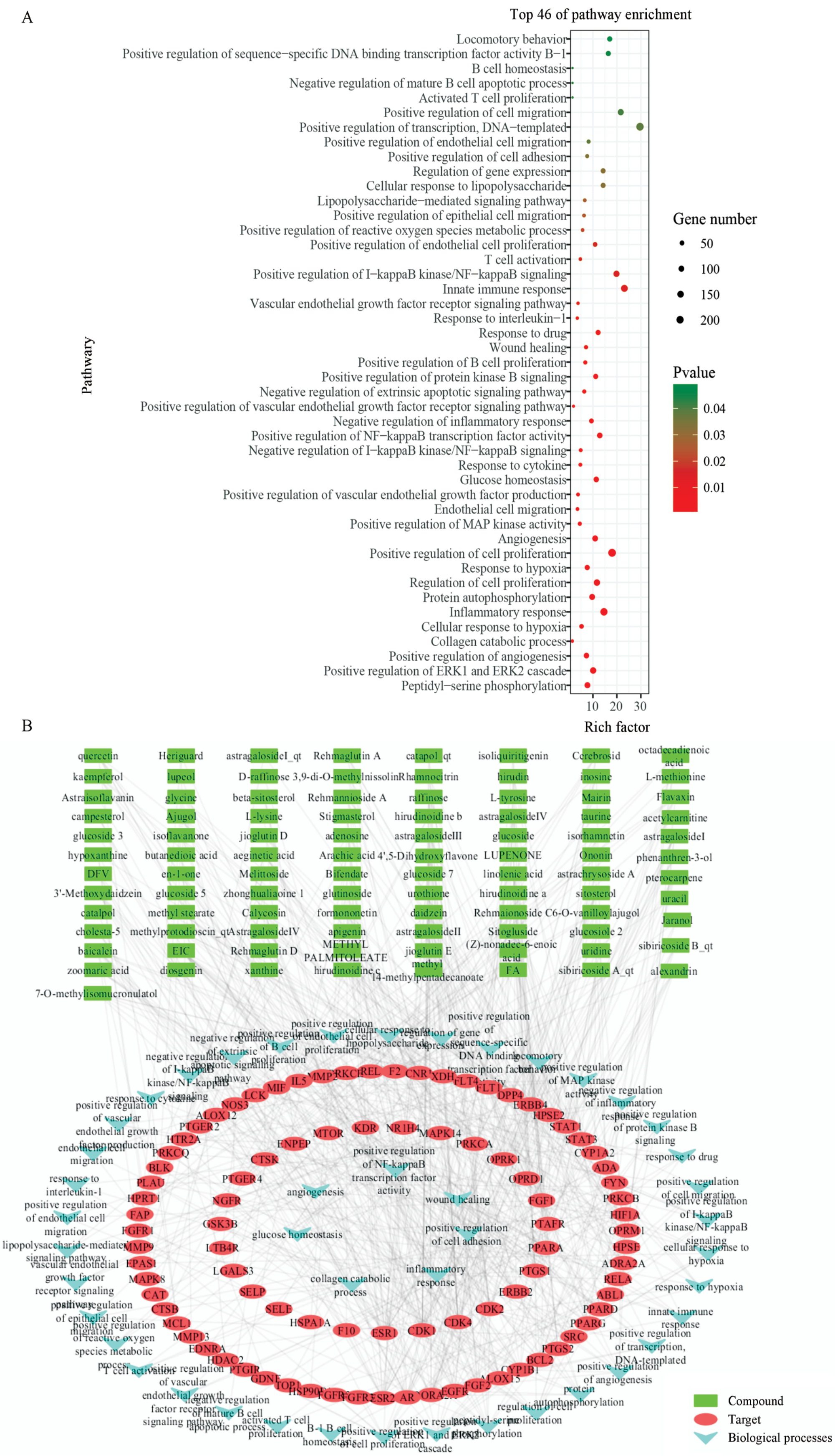

Figure 5　The related targets and biological processes of QZHGT-associated active compounds involved in the roles of hypoglycemic and wound healing. A: Active compounds-involved biological processes; B. Compound-target-biological processes network

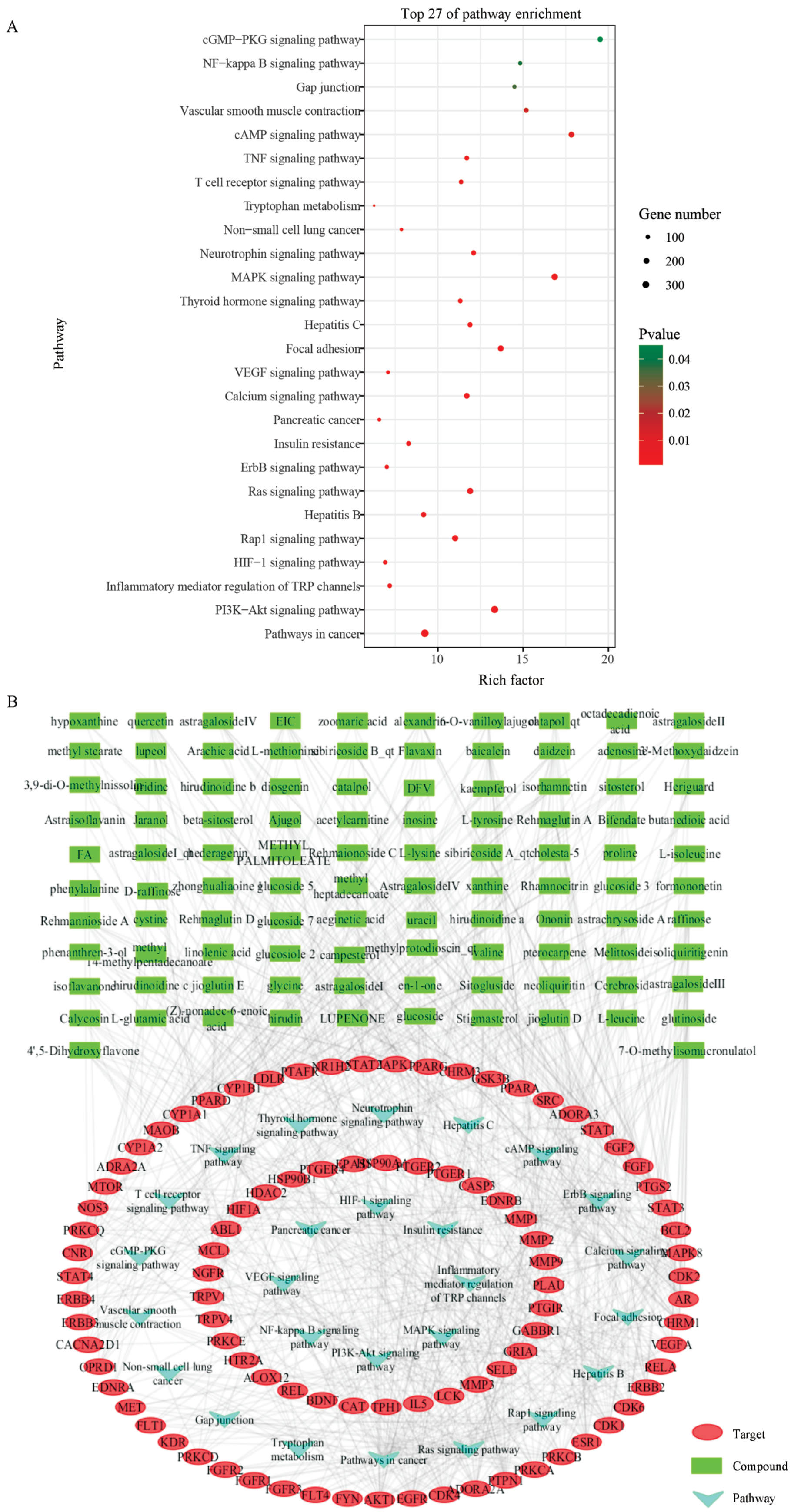

Figure 6 The related targets and signaling pathways of QZHGT-associated active compounds involved in the roles of hypoglycemic and wound healing. A: Active compounds-involved signaling pathways; B: Compound-target-pathway network

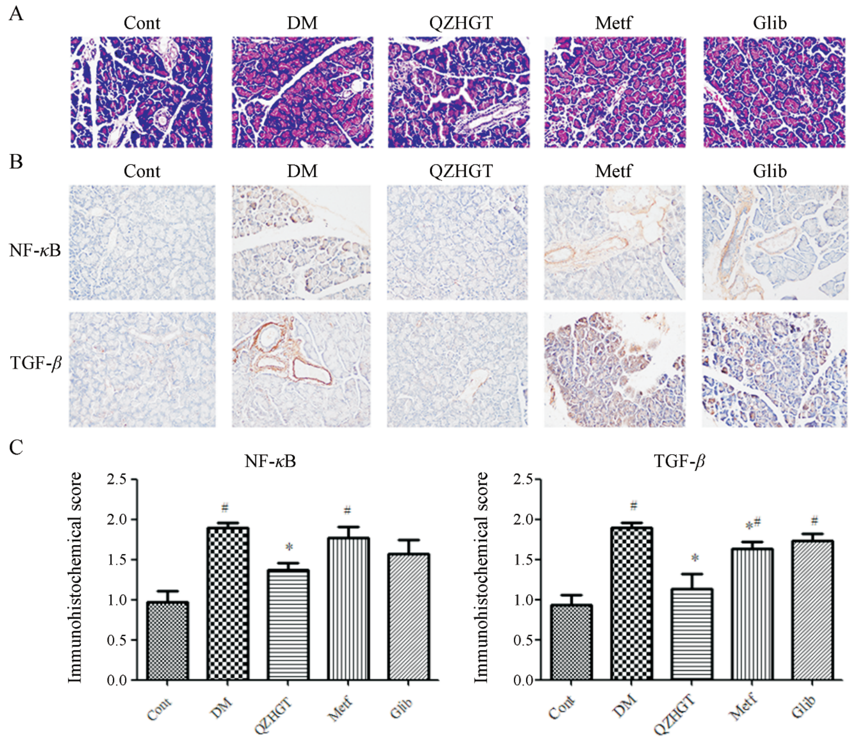

Figure 7 QZHGT prevents islet injury in diabetic rats. A: HE staining shows that QZHGT prevents islet injury in diabetic rats; B: Immunohistochemistry reveals that QZHGT decreases the expression of NF-κB and TGF-β in islet of diabetic rats; C: Semi-quantitative immunohistochemical score of NF-κB and TGF-β. n = 18, $\bar{x} \pm s$. $^{*}P$<0.05 *vs* DM; $^{\#}P$<0.05 *vs* Cont

食、多尿等作用明显, 对上述高凝状态无明显影响, 提示糖尿病高凝状态不仅仅与血糖有关, 单单降低血糖不能阻止糖尿病足病。芪蛭降糖片虽然降糖作用出现较晚, 但能明显改善糖尿病高凝状态, 与“益气活血化瘀”中医治疗糖尿病足溃疡的“通畅气血、去腐生肌”治疗理念相符。

网络药理学是通过系统方法分析药物对生物网络的作用和影响、从整体角度认识和预测药物靶点的方法, 非常适用于解释中药单复方复杂成分的作用机制[17]。本研究采用网络药理学方法,构建了芪蛭降糖片的成分−靶点−通路网络, 分析了芪蛭降糖片防治糖尿病足溃疡的作用机制。在本研究中, 成分−靶点−疾病网络有289个节点, 2 586条连接。通过筛选核心网络有162个节点, 1 778条连接, 涉及的生物过程有46个, 信号通路富集27条上, 其中辅助降糖相关靶点涉及的生物过程可能主要是葡萄糖稳态、B细胞增殖的正向调节、B-1 B细胞稳态等; 改善糖尿病足相关的靶点涉及的生物过程可能主要是血管生成的正向调节、胶原分解代谢过程、炎症反应、NF-κB转录因子活性的正向调节、伤口愈合和细胞黏附的正向调节等。辅助降糖相关靶点涉及的信号通路可能主要是胰岛素抵抗、胰腺癌; 改善糖尿病血凝状态相关靶点涉及的信号通路是HIF-1信号通路; 改善糖尿病足相关靶点涉及的信号通路是PI3K-Akt信号通路、TRP通道的炎症介质调节、VEGF信号通路、MAPK信号通路和NF-κB信号通路等, 每个生物过程和信号传导调节参与的活性成分都在3个以上, 充分体现了芪蛭降糖片多成分、多靶点和多通路之间整合调节作用的特点。iNOS是胰岛分泌受损和β细胞死亡的关键调控因子[18], VEGF在胰岛发育和损伤过程中起着重要作用[19], 机制预测显示芪蛭降糖片多个成分靶向胰岛素抵抗通路, 与本研究显示的芪蛭降糖片能够降低iNOS和VEGF血清水平结果一致。链脲佐菌素激活NF-κB信号通路是诱导iNOS信号传导的关键[20], 同时NF-κB信号通路激活也能够造成β细胞功能障碍和胰岛损伤[21]。TGF-β为一类多功能生长因子和MAPK信号通路的上游调控蛋白, 其激活过表达也是组织纤维化的标志[22]。本研究根据机制预测的芪蛭降糖片多个成分可靶向NF-κB和MAPK信号通路, 检测了胰腺组织中NF-κB和TGF-β的表达, 结果显示芪蛭降糖片显著抑制其在胰腺组织中的高表达, 验证了机制预测中芪蛭降糖片有保护胰岛损伤的可能性, 也为芪蛭降糖片辅助降糖作用提供了理论依据。糖尿病患者高凝状态是组织损伤修复延迟的主要原因之一[23], HIF-1与机体高凝状态密切相关[24], 机制预测提示芪蛭降糖片多个成分靶向HIF-1信号通路, 这可以解释本研究芪蛭降糖片能够改善糖

尿病大鼠高凝状态的原因。另外, VEGF通路、MAPK通路和NF-κB通路均与组织损伤过程密切相关[25-29], 芪蛭降糖片中多个活性成分对这些通路信号传导有调节作用, 应该是芪蛭降糖片能显著改善糖尿病足溃疡的主要原因。

综上所述, 芪蛭降糖片能通过多途径、多靶点阻止糖尿病及其足溃疡, 长期服用有改善微循环和促进创伤修复作用, 是前景非常好的糖尿病辅助治疗及防治糖尿病足病的药物。

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