

绞股蓝皂苷联合银杏叶提取物对2型糖尿病合并NAFLD大鼠脂肪肝程度的影响

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摘要: 目的 观察绞股蓝皂苷(GPS)和银杏叶提取物(GBE)混合物对2型糖尿病(T2DM)合并非酒精性脂肪性肝病(NAFLD)大鼠脂肪肝程度的影响。方法 40只SPF级雄性SD大鼠随机分为空白组、T2DM合并NAFLD模型组,造模周期8周。将T2DM合并NAFLD模型随机分为模型组(给予同等体积的纯净水灌胃)、对照组[给予GPS 0.5 g/(kg·d)灌胃]、治疗组[给予GPS 0.5 g/(kg·d)加 GBE 0.1 g/(kg·d)灌胃]。继续予高糖高脂饲料,自由饮水;治疗周期为6周;实验周期14周。检测血糖(BS)、甘油三酯(TG)、总胆固醇(TC)、ALT、AST。检测肝组织TG及肝脏病理学。结果 空白组、模型组、治疗组、对照组BS水平分别为(5.50 ± 0.51) mmol/L、(18.76 ± 4.20) mmol/L、(10.78 ± 3.38) mmol/L、(14.62 ± 3.32) mmol/L,治疗组、对照组低于模型组($P < 0.01$),治疗组低于对照组($P < 0.01$)。空白组、模型组、治疗组、对照组TG水平分别为(0.81 ± 0.11) mmol/L、(1.29 ± 0.14) mmol/L、(0.90 ± 0.09) mmol/L、(1.09 ± 0.10) mmol/L,治疗组、对照组低于模型组($P < 0.01$),治疗组低于对照组($P < 0.05$)。空白组、模型组、治疗组、对照组TC水平分别为(1.39 ± 0.13) mmol/L、(3.69 ± 0.27) mmol/L、(2.59 ± 0.19) mmol/L、(3.11 ± 0.18) mmol/L,治疗组、对照组低于模型组($P < 0.01$),治疗组低于对照组($P < 0.01$)。空白组、模型组、治疗组、对照组ALT水平分别为(35.12 ± 3.34) U/L、(60.68 ± 5.12) U/L、(42.13 ± 4.99) U/L、(50.12 ± 4.68) U/L,治疗组、对照组低于模型组($P < 0.01$),治疗组低于对照组($P < 0.05$)。空白组、模型组、治疗组、对照组AST水平分别为(81.15 ± 7.14) U/L、(168.12 ± 10.49) U/L、(112.23 ± 10.12) U/L、(139.12 ± 11.68) U/L,治疗组、对照组低于模型组($P < 0.01$),治疗组低于对照组($P < 0.01$)。空白组、模型组、治疗组、对照组肝组织TG水平分别为(30.26 ± 2.48) mg/G、(228.46 ± 8.48) mg/G、(153.12 ± 9.98) mg/G、(196.24 ± 9.78) mg/G,治疗组、对照组低于模型组($P < 0.05$),治疗组低于对照组($P < 0.01$)。空白组为正常肝组织,模型组为重度脂肪肝,治疗组和对照组为中-重度脂肪肝,脂肪肝程度治疗组轻于对照组。结论 GPS和GBE混合物通过调整血糖、脂质代谢,减少肝组织TG沉积,保护肝细胞,达到降低T2DM合并NAFLD大鼠模型脂肪肝程度的目的。

关键词: 2型糖尿病合并非酒精性脂肪性肝病;绞股蓝提取物和银杏叶提取物混合物;脂肪肝程度;影响;动物

Effect of the degrees of fatty liver disease in rats with type 2 diabetes mellitus and nonalcoholic fatty liver disease was treated by gypenosides and ginkgo biloba extract mixture

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Abstract: Objective To observe effect of the degrees of fatty liver disease in rat with type 2 diabetes mellitus and nonalcoholic fatty liver disease was inhibited by Gypenosides and Ginkgo Biloba Extract Mixture. **Methods** Total of 40 SPF male SD rats, body mass 220-250 g, were randomly divided into blank control group, model group with type two diabetes mellitus and nonalcoholic fatty liver disease. After eight weeks the NAFLD model in rats was made, model group were divided into three groups: treatment group were perfused with 500 mg/(kg·d)

GPS and 0.1g/(kg·d) ginkgo biloba extract mixture, control group were perfused with 500 mg/(kg·d) GPS, model group were perfused with the same volume of water, and high fat diet at the same time, the experimental period was six weeks. The experimental period was 14 weeks. Blood sugar, triglycerides, total cholesterol (TC), ALT, AST in the plasma in rat was test. It was tested that triglycerides in the liver tissue. The Liver tissue was detected by pathology. **Results** BS levels in plasma: blank control group (5.50 ± 0.51) mmol/L, model group (18.76 ± 4.20) mmol/L, treatment group (10.78 ± 3.389) mmol/L, control group (14.62 ± 3.32) mmol/L, treatment group and control group was lower than model group, there was a significant difference ($P < 0.01$), treatment group was lower than control group, there was a significant difference ($P < 0.05$). TG levels in plasma: blank control group (0.81 ± 0.11) mmol/L, model group (1.29 ± 0.14) mmol/L, treatment group (0.90 ± 0.09) mmol/L, control group (1.09 ± 0.10) mmol/L, treatment group and control group was lower than model group, there was a significant difference ($P < 0.01$), treatment group was lower than and control group, there was a significant difference ($P < 0.05$). TC levels in plasma: blank control group (1.39 ± 0.13) mmol/L, model group (3.69 ± 0.27) mmol/L, treatment group (2.59 ± 0.19) mmol/L, control group (3.11 ± 0.18) mmol/L, treatment group and control group was lower than model group, there was a significant difference ($P < 0.05$), treatment group was lower than control group ($P < 0.05$). ALT levels in plasma: blank control group (35.12 ± 3.34) U/L, model group (60.68 ± 5.12) U/L, treatment group (42.13 ± 4.99) U/L, control group (50.12 ± 4.68) U/L, treatment group and control group was lower than model group, there was a significant difference ($P < 0.05$), treatment group was lower than control group ($P < 0.05$). AST levels in plasma: blank control group (81.15 ± 7.14) U/L, model group (168.12 ± 10.49) U/L, treatment group (112.23 ± 10.12) U/L, control group (139.12 ± 11.68) U/L, treatment group and control group was lower than model group ($P < 0.01$), treatment group was lower than control group ($P < 0.01$). TG levels in liver tissue: blank control group (30.26 ± 2.48) mg/g, model group (228.46 ± 8.48) mg/g, treatment group (153.12 ± 9.98) mg/g, control group (196.24 ± 9.78) mg/g, treatment group and control group was lower than model group, there was a significant difference ($P < 0.05$), treatment group was lower than control group ($P < 0.05$). Liver pathology: blank control group was normal liver tissues. Model group was severe fatty liver disease. Treatment group and control group was moderate to severe fatty liver disease, and treatment group was lighter than control group. **Conclusions** The degree of T2DM and nonalcoholic fatty liver disease in rats was inhibited by gypenosides and ginkgo biloba extract mixture, through blood sugar and lipid metabolism adjusted, and reduce the liver tissue TG deposition, protecting liver cells.

Key words: T2DM and nonalcoholic fatty liver disease; GPS and GBE mixture; Degree of fatty liver disease; Influence; Animal

现代生活方式, 非酒精性脂肪性肝病 (nonalcoholic fatty liver disease, NAFLD) 和2型糖尿病 (type 2 diabetes mellitus, T2DM) 发病率急剧上升。NAFLD通过非酒精性脂肪性肝炎 (NASH) 发展为NASH相关肝硬化、肝癌^[1,2]; NAFLD导致高脂血症、动脉粥样硬化、T2DM^[3-5]; T2DM患者NAFLD检出率为50.45%^[6]。T2DM并NAFLD导致的恶性循环对健康的影响巨大, 其干预研究一直是国内外研究的热点。既往研究发现绞股蓝皂苷 (gypenosides, GPS) 对T2DM合并NAFLD具有治疗作用^[7-9], 银杏叶提取物 (ginkgo biloba extract, GBE) 对NAFLD和T2DM并NAFLD有治疗作用^[10,11]。本研究以GPS为对照药物, 观察GPS和GBE混合物对T2DM合并NAFLD大鼠脂肪肝程度的影响, 为临床提供治疗选择。

1 材料与方法

1.1 实验动物与场地 健康雄性SPF级SD (Sprague-

Dawley, SD) 大鼠40只, 体质量220~250 g, 湖北医药学院实验动物中心提供 (实验动物合格证书: 00011597), 实验场地湖北省十堰市实验动物中心 (湖北省实验动物设施使用证明号: 00016036)。

1.2 实验饲料 普通饲料由湖北医药学院动物实验中心提供。胆固醇购自北京双旋生物培养基制品厂。猪油由市售板油自行炼制而成, 蛋黄粉由市售鸡蛋自行制作。高脂饲料按照课题组配方 (配方: 82.5%普通饲料+ 2.5%胆固醇+ 8%蛋黄+ 7%猪油)^[12], 由湖北医药学院动物实验中心配制。

1.3 材料与设备 甘油三酯 (triglycerides, TG)、总胆固醇 (total cholesterol, TC)、AST、ALT检测试剂为瑞士Roche/Hitache公司原装进口试剂。全自动生化检测仪 (瑞士产, Roche/Hitache7600)。冷冻低温离心机 (上海中科生物医学高科技开发有限公司, DL-45R-L)。德国产组织匀浆机。GPS

购自安康东科麦迪森天然药业有限公司, 纯度为98%。GBE购自徐州恒凯银杏制品有限公司(中国药典2005版银杏叶提取物)。

1.4 实验模型的建立

1.4.1 实验动物的分组 参照文献^[7-9], SD大鼠购入后, 普通饲料饲养观察1周, 动物无死亡及不良反应。按照实验设计将40只大鼠分为空白组(7只), T2DM合并NAFLD模型组(33只)。

1.4.2 T2DM合并NAFLD造模与干预及空白组饲养与管理 参照文献^[8,9], 每鼠每天高糖高脂饲料20 g左右, 分两次投入, 喂养4周; 大鼠隔夜空腹腹腔注射链尿佐菌素(streptozotocin, STZ) 40 mg/kg, 48小时后尾静脉取血, 血糖 > 11.1 mmol/L, 纳入实验组, 造模成功率为100%; 继续给予高糖高脂饲料喂养至8周, 建立T2DM合并NAFLD模型。造模期间大鼠死亡3只。将30只大鼠分为对照组[给予GPS 0.5 g/(kg·d)灌胃]、治疗组[给予GPS 0.5 g/(kg·d)联合GBE 0.1 g/(kg·d)]、模型组(给予同等体积的纯净水灌胃)各10只, 继续给予高糖高脂饲料, 自由饮水; 治疗周期为6周; 实验周期14周。治疗期间模型组大鼠死亡1只, 治疗组及对照组大鼠无死亡。空白组每天给予普通饲料约20 g(根据NAFLD组进食量调整), 饲料分2次投入。自由饮水。实验周期14周。

1.5 血液标本采集与检测 饲养至14周末, 末次给药后禁食12小时, 用35 g/L的戊巴比妥钠(35 g/kg)腹腔注射麻醉, 腹主动脉取血, 低温离心, 取血浆, -20°C 保存。按照设备和试剂说明书, 检测血糖(BS)、TG、TC、ALT、AST, 采用全自动生化分析仪测定。

1.6 肝脏标本的采集与检测

1.6.1 肝脏病理学 在肝脏最大叶边缘内0.5 cm, 取肝组织1份, 常规固定、石蜡包埋、切片, HE染色、普通显微镜检测。

1.6.2 肝组织匀浆的制备 按照文献^[7-9]在肝脏最大叶边缘0.5 cm处取小块肝组织1块, 冰生理盐水冲洗, 滤纸吸干, 称取0.5 g置于预冷的5 ml生理盐水, 迅速用内切式匀浆机4000 r/min匀浆, 10秒/次, 间隙30秒, 连续3次, 碎冰块中制成10%的肝匀浆, 高

速冷冻离心机 4°C 、3000 r/min离心10分钟, 取上清3 ml密封, -20°C 冷存待检。

1.6.3 肝组织匀浆TG测定 按照文献^[7-9]方法及设备和试剂操作常规进行。

1.7 统计学处理 应用SPSS 17.0软件进行统计学分析, 计量资料采用 $\bar{x} \pm s$ 表示, 组间比较采用 t 检验, 检验水准 $\alpha = 0.05$ 。

2 结果

2.1 血糖水平比较 与模型组比较, 治疗组、对照组血糖水平明显下降($P < 0.01$), 治疗组低于对照组, 比较有显著差异($P < 0.05$), 见表1。

2.2 血TG、TC水平比较 与模型组比较, 治疗组、对照组血TG、TC水平明显下降($P < 0.05$), 且治疗组低于对照组($P < 0.05$), 差异有统计学意义, 见表1。

2.3 ALT、AST水平比较 与模型组比较, 治疗组、对照组血ALT、AST水平明显下降($P < 0.01$), 治疗组低于对照组($P < 0.05$), 差异有统计学意义。

2.4 肝组织TG水平比较 与模型组比较, 治疗组、对照组肝组织TG含量均明显降低($P < 0.01$), 治疗组与对照组比较, 差异有统计学意义($P < 0.05$)。

2.5 肝组织病理学 空白组为正常肝组织, 模型组为重度脂肪肝, 治疗组和对照组为中-重度脂肪肝, 治疗组脂肪肝程度轻于对照组, 见图1。

3 讨论

NAFLD由于其对全身和肝脏局部的影响一直受到肝病学界和内分泌科的关注, 成为跨学科研究的热点之一。NAFLD发病机制复杂, 需要多靶点干预, 中医药由于其多靶点作用在NAFLD的防治上占有重要位置。T2DM与NAFLD互相促进、互为因果, 对健康影响巨大。本研究通过血ALT、AST、TG、TC以及肝组织TG沉积量、肝组织病理学比较, 观察GPS和GBE混合物对T2DM并NAFLD的治疗作用。

既往研究证实, GPS对T2DM合并NAFLD疗效确切^[7-9], 本研究以GPS作对照药物, 观察GPS和GBE混合物的对T2DM合并NAFLD疗效。发现GPS和GBE混合物治疗组干预可以明显降低T2DM合并NAFLD

表 1 4组大鼠血TG、TC、AST、ALT及肝组织TG检测结果 ($\bar{x} \pm s$)

组别	BS (mmol/L)	TG (mmol/L)	TC (mmol/L)	ALT (U/L)	AST (U/L)	肝组织TG (mg/g)
空白组 (n=7)	5.50 ± 0.51	0.81 ± 0.11	1.39 ± 0.13	35.12 ± 3.34	81.15 ± 7.14	30.26 ± 2.48
模型组 (n=9)	18.76 ± 4.20 ^{ab}	1.29 ± 0.14 ^{ac}	3.69 ± 0.27 ^{ac}	60.68 ± 5.12 ^{ac}	168.12 ± 10.49 ^{ac}	228.46 ± 8.48 ^{ac}
治疗组 (n=10)	10.78 ± 3.38 ^b	0.90 ± 0.09 ^c	2.59 ± 0.19 ^b	42.13 ± 4.99 ^c	112.23 ± 10.12 ^b	153.12 ± 9.98 ^c
对照组 (n=10)	14.62 ± 3.32	1.09 ± 0.10	3.11 ± 0.18	50.12 ± 4.68	139.12 ± 11.68	196.24 ± 9.78

注: BS: 血糖。与治疗组比较, ^a $P < 0.01$; 与对照组比较, ^b $P < 0.01$, ^c $P < 0.05$

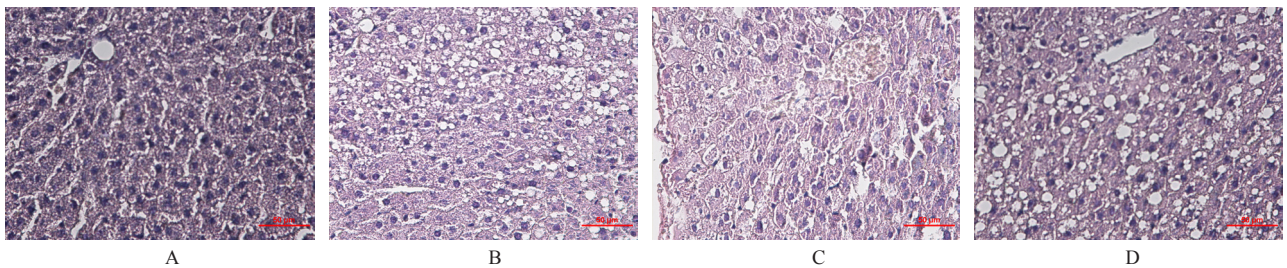


图 1 不同组脂肪肝程度变化 (HE染色, 400×)

注: A为空白组; B为模型组; C为治疗组; D为对照组

大鼠血糖、TG、TC、ALT及AST水平,降低肝组织TG沉积量,减轻脂肪肝程度,疗效优于GPS。

GPS的基础研究一直是中医药防治代谢性疾病研究的热点之一^[11,12]。Norberg等^[13]从绞股蓝中分离提纯出一种新的皂苷,存在4种可以相互转化的立体异构体,在体外及动物实验中均发现,其可刺激胰岛细胞释放胰岛素,且呈现剂量依赖性。Xu等^[14]对绞股蓝中分离得到的皂苷及其衍生物的降糖作用进行了研究,发现其可以通过抑制蛋白酪氨酸酯酶来控制胰岛素的敏感度,说明GPS通过刺激胰岛素分泌、提高胰岛素敏感性、调节血糖代谢、调节血脂代谢抑制“第一次打击”,通过抗纤维化^[15]、抑制脂质过氧化、抗感染抑制“第二次打击”。GBE具有调整高脂血症大鼠模型血脂代谢的作用^[16],对脂肪肝的治疗作用已经得到公认^[17]。本研究发现GPS和GBE混合物可以发挥比单药更好的疗效。既往有研究^[17]证明,由绞股蓝和银杏叶组成的野生绞股蓝复方具有良好的治疗脂肪肝作用,无配伍禁忌。而且,GPS提取物和GBE提取物还具有携带方便的优点,值得临床推广应用。

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