

复方鳖甲软肝方防治大鼠肾间质纤维化作用机理的实验研究*

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摘 要:目的:采用单侧输尿管结扎(UUO)肾间质纤维化模型,探讨复方鳖甲软肝方对肾间质纤维化大鼠血生化及肾组织病理形态学的影响。方法:将大鼠随机分为7组,正常组、假手术组、模型组、苯那普利组和复方鳖甲软肝方低、中、高剂量组。UUO术后4周处死大鼠,并应用HE、六胺银染色及Mallory染色,观察肾组织病理学改变。结果:模型组肾小管结构严重破坏,管腔塌陷,上皮细胞大量坏死;有些部位肾小管和间质的结构基本消失,间质中出现弥漫性细胞增殖,并广泛间质纤维化。复方鳖甲软肝方组和苯那普利组纤维化病理改变明显减轻,其中以复方鳖甲软肝方中剂量组作用明显。结论:复方鳖甲软肝方可减少炎细胞的浸润、减轻肾小管的损伤,进而延缓肾间质纤维化的病程进展。

关键词:单侧输尿管结扎 复方鳖甲软肝方 肾间质纤维化 作用机理

肾脏疾病进展到慢性肾衰竭的确切发病机制至今尚未完全阐明,但其共同的通路却是肾脏萎缩、纤维化^[1]。肾脏纤维化包括肾小球硬化和肾间质纤维化,由于肾小管与间质在解剖学上特点后者简称为肾间质纤维化。近年来的研究发现,各种原发性肾脏疾病引起的肾排泄功能损害与肾小管间质病变程度密切相关,肾小管间质的病变特点为炎症细胞的浸润,肾小管萎缩和间质纤维化^[2~3]。因此,防治肾间质纤维化是延缓肾功能恶化的主要措施之一。

近年来,中医药在防治器官纤维化方面取得了

较好的效果,并且显示出良好的应用前景。复方鳖甲软肝方具有活血化瘀、软坚散结、益气养阴、清热解毒之功效。根据“异病同治”理论^[4],为了探讨该方对肾间质纤维化的治疗作用,我们建立单侧输尿管结扎肾间质纤维化大鼠模型,研究该方对肾间质纤维化大鼠肾组织病理形态的影响,以期为该方防治肾间质纤维化提供实验依据。

一、材料与方法

1. 动物

雄性Wistar大鼠105只,体重 200 ± 20 g,由中国医学科学院实验动物中心提供。

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2. 药物

复方鳖甲软肝方(以下简称 FFBJRGF)[内蒙古福瑞制药有限公司提供,批号 20020501],苯那普利[北京诺华制药公司,批准文号:国药准字 H20000292]。

3. 模型建立与分组

105 只大鼠,适应性喂养 1 周,随机取 12 只作为正常组,其余作为实验组。① UUO 模型建立^[5]:大鼠水合氯醛麻醉后取俯卧位,切开右侧背部皮肤至腹膜后,游离肾脏及输尿管,在靠近肾下极处结扎并离断输尿管后,逐层缝合皮肤。假手术组除不结扎及离断输尿管外,余同上述方法。术中大鼠死亡 1 只,术后将大鼠按体重随机分为 6 组。② 分组及用药:正常组 12 只;假手术组 12 只;模型组 15 只;苯那普利组 15 只;FFBJRGF 小、中、大剂量组各 15 只;药物组灌胃给药,其余各组给予等量生理盐水。小、中、大剂量大鼠用量分别为人用量单位体重的 3.5、7、14 倍。以上各组动物分笼饲养,给予普通饲料及自由饮水,实验用药 4 周。

4. 24 小时尿收集方法及检测指标

第 27d 时,收集 24h 尿。

测尿 NAG 酶和 β_2 微球蛋白。

5. 血清学指标测定方法

第 28d 时,取大鼠颈总动脉血,离心分离血清,测定 BUN、Scr 的含量

6. 肾组织病理学检查

将所取的左肾部分肾用 10% 中性福尔马林固定,经脱水浸蜡后,进行石蜡包埋、切片,切片厚度为 4 μ m。将组织病理切片按常规方法进行 HE、六胺银染色及 Mallory 染色。

7. 实验数据统计学处理

各组大鼠实验结果采用 $\bar{x} \pm SD$ 表示,所有实验数据均采用统计软件 SPSS11.0 进行单因素方差分析。

二、结 果

1. 复方鳖甲软肝方对 UUO 各组大鼠体重、肾重及肾重体重比的影响

与正常组、假手术组大鼠肾重体重比比较,模型组、苯那普利组、FFBJRGF 高、中、低剂量组大鼠肾重体重比增加,显著差异($p < 0.01$)。与模型组大鼠肾重体重比比较,苯那普利组、FFBJRGF 高、中、低剂量组大鼠肾重体重比减少,有显著或极显著差异($p < 0.05$ 或 $p < 0.01$)。

2. 尿 NAG 酶和 β_2 微球蛋白的测定

与正常组、假手术组大鼠血清相比,28d 时模型组大鼠尿 NAG 酶和 β_2 -mG 含量明显升高($p < 0.05$)。FFBJRGF 高、中、低剂量均可降低肾间质纤维化大鼠尿 NAG 酶和 β_2 -mG 的含量,其中 FFBJRGF 中剂量对尿 NAG 酶和 β_2 -mG 的含量的降低作用较为显著($p < 0.05$)。

3. 大鼠血清 BUN 及 Scr

与正常组、假手术组大鼠血清相比,28d 时模型

表 1 大鼠肾体重、肾重及肾重体重比

组别	N	体重	肾重	肾重体重比
正常组	12	312.5 $\pm$ 26.05	1.3055 $\pm$ 0.22774	4.1848 $\pm$ 0.75473
假手术组	10	313.5 $\pm$ 19.50	1.2123 $\pm$ 0.19696	3.8826 $\pm$ 0.69590
模型组	12	305.0 $\pm$ 18.61	15.4726 $\pm$ 3.05436 Δ	50.9708 $\pm$ 10.88851 Δ
苯那普利组	15	312.9 $\pm$ 31.33	10.7380 $\pm$ 3.18241 Δ	34.5099 $\pm$ 10.02769 Δ
FFBJRGF 低剂量组	14	304.0 $\pm$ 17.24	11.1053 $\pm$ 3.34886 Δ	36.6752 $\pm$ 10.93965 Δ
FFBJRGF 中剂量组	14	324.5 $\pm$ 12.56	11.3102 $\pm$ 1.61584 Δ	39.3193 $\pm$ 8.19538 Δ
FFBJRGF 高剂量组	15	315.6 $\pm$ 19.81	10.9629 $\pm$ 3.59426 Δ	34.5983 $\pm$ 10.27265 Δ

注: Δ 与正常组及假手术组相比 $P < 0.05$, Δ 模型组与用药组相比 $P < 0.05$ 。

表 2 大鼠尿 NAG 酶及 β_2 微球蛋白含量比较($\bar{x} \pm SD$)

组别	N	NAG(U/L)	β_2 -MG(μ g/L)
正常组	10	7.3450 $\pm$ 1.82587	35.14 $\pm$ 4.26
假手术组	10	6.9630 $\pm$ 1.16925	34.90 $\pm$ 3.59
模型组	10	18.3300 $\pm$ 1.43257 Δ	95.82 $\pm$ 5.32 Δ
苯那普利组	10	13.2660 $\pm$ 1.52729 Δ	55.15 $\pm$ 3.55 Δ
FFBJRGF 高剂量组	10	16.0470 $\pm$ 1.58614 Δ	64.76 $\pm$ 3.78 Δ
FFBJRGF 中剂量组	10	13.9430 $\pm$ 1.34415 Δ	54.66 $\pm$ 3.69 Δ
FFBJRGF 低剂量组	10	16.4080 $\pm$ 2.19322 Δ	59.32 $\pm$ 4.92 Δ

注: Δ 与正常组相比 $P < 0.05$, Δ 与模型组相比 $P < 0.05$ 。

表3 大鼠血清中BUN及Scr的含量比较($\bar{X} \pm SD$)

组别	N	Scr($\mu\text{mol/L}$)	BUN(mmol/L)
正常组	12	76.655 $\pm$ 3.908398	6.419167 $\pm$ 0.331398
假手术组	10	88.899 $\pm$ 3.414532	6.4490 $\pm$ 0.483911
模型组	12	110.7117 $\pm$ 3.522948 [△]	7.990833 $\pm$ 0.378805 [△]
苯那普利组	15	96.394 $\pm$ 3.734192 ^{△▲}	6.419167 $\pm$ 0.275498 ^{△▲}
FFBJRGF高剂量组	14	98.77429 $\pm$ 3.16477 ^{△▲}	7.495714 $\pm$ 0.16066 ^{△▲}
FFBJRGF中剂量组	14	95.37643 $\pm$ 2.605062 ^{△▲}	7.145714 $\pm$ 0.245529 ^{△▲}
FFBJRGF低剂量组	15	98.61867 $\pm$ 2.238947 ^{△▲}	7.176667 $\pm$ 0.19953 ^{△▲}

注: Δ 与正常组及假手术组相比 $P < 0.05$, $\blacktriangle$ 与模型组相比 $P < 0.05$ 。

组大鼠血清中BUN及Scr的含量明显升高($p < 0.05$)。FFBJRGF高、中、低剂量均可降低肾间质纤维化大鼠血清中BUN及Scr的含量,其中FFBJRGF中剂量对BUN及Scr的含量的降低作用显著($p < 0.05$)。

4. 病理组织学检查

(1) 大体观察。

解剖后肉眼观察正常组和假手术组左侧肾脏大小正常,色泽正常,表面光滑弹性好。模型组大鼠左侧肾脏明显增大,皮质明显变薄,呈囊状,颜色暗红,表面粗糙不平,有囊肿和瘢痕。苯那普利组、FFBJRGF高剂量组、FFBJRGF中剂量组、FFBJRGF低剂量组大鼠左侧肾脏增大,皮质变薄,呈囊状,颜色略暗,表面粗糙不平,有囊肿和瘢痕。说明FFBJRGF在不同程度上可以改善单侧输尿管结扎肾间质纤维化病理损害。

(2) 光镜检查。

HE染色:正常组和假手术组肾小球、肾小管结构正常;模型组肾小管扩张,肾小管上皮细胞脱落、空泡变性;肾小管间质大量炎细胞浸润,肾间质纤维化,肾小管和间质的结构基本消失;严重者肾间质纤维化,而肾小球病变轻微。与模型组比较,FFBJRGF小、中、大剂量组有明显减轻,肾小管腔轻度扩张,上皮细胞完整;间质轻度炎细胞浸润减少,与苯那普利组比较,其中FFBJRGF中剂量组病变最轻。见图1~图8。

Mallory染色:正常组和假手术组肾脏胶原染色主要位于肾小管基膜、肾小囊、系膜区和肾

小管间的毛细血管周围,而肾小管周围间质染色较少。与假手术组比较,模型组肾间质明显增宽,间质细胞增多,胶原成分显著增加。与模型组比较,FFBJRGF组肾间质纤维化程度较模型组和苯那普利组明显降低。与苯那普利组比较,中剂量组胶原沉积明显减少。见图9~图16。

六胺银染色:正常组和假手术

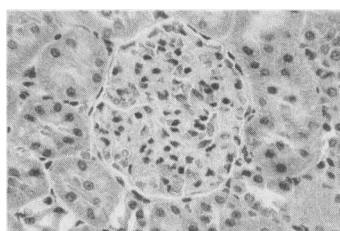


图1 正常组大鼠肾组织,正常结构肾小球、肾小管,HE $\times 400$

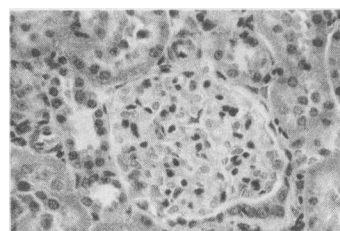


图2 假手术组大鼠肾组织,正常结构肾小管,HE $\times 400$

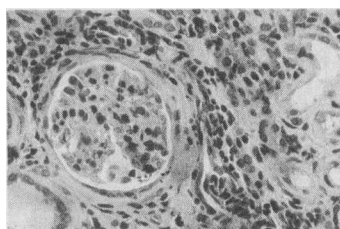


图3 模型组大鼠肾组织,肾小管扩张,上皮细胞脱落,肾小管基底膜不完整;肾小管间质大量炎细胞浸润、间质纤维化,结构破坏,系膜细胞轻度增生,HE $\times 400$

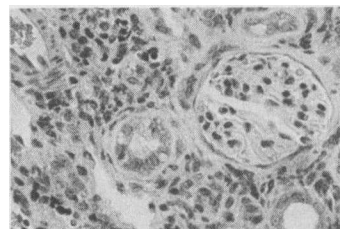


图4 模型组大鼠肾组织,肾小管上皮细胞空泡样变,肾小管萎缩;间质大量炎细胞浸润,间质纤维化;肾小球结构基本正常,HE $\times 400$

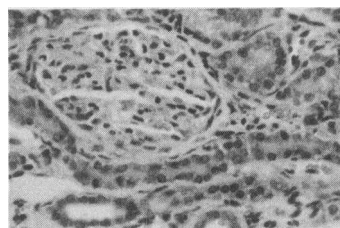


图5 苯那普利组大鼠肾组织,肾小管腔轻度扩张,上皮细胞完整;间质轻度炎细胞浸润;肾小球结构正常,HE $\times 400$

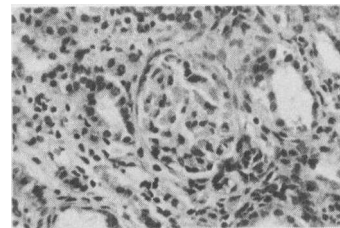


图6 复方鳖甲软肝方小剂量组大鼠肾组织,肾小管轻度扩张,上皮细胞脱落;间质有较多炎细胞浸润,间质中度纤维化;球囊壁粘连,轻度系膜细胞增生,HE $\times 400$

组肾小球、肾小管基底膜完整,围绕肾小球和肾小管周围。与假手术组比较,模型组有的肾小管基底膜增粗,有的内折,而肾小球基底膜几乎没有改变。复方鳖甲软肝方组和苯那普利组肾小管基底膜增粗、内折现象有所减少。

三、讨 论

近年来研究发现肾小球肾炎病人的肾小球滤过率(GRF)下降与肾小管萎缩程度密切相关,而与肾小球病理严重程度并不相关。各种肾病在向慢性肾

衰竭的发展过程中,有着共同的途径—肾小管萎缩,肾间质纤维化^[6]。在各种原发、继发肾小球疾病和非肾小球疾病中,肾小管间质病变程度是反映肾功能下降严重程度和判断预后最重要的指标。

复方鳖甲软肝方是一种治疗肝纤维化及早期肝硬化的三类中药新药。中医认为肝纤维化和肾纤维化,虽然有各自的病机特点,但又有内在的共同发病规律。在发病及演变过程中,正气不足为本病机之本,兼夹实邪乃本病机之关键,“湿”、“毒”、“虚”、“瘀”贯穿疾病的始终^[4]。现代研究表明,鳖甲具有提高机体免疫力^[7],降低水肿,抗炎,抑制成纤维细胞增生,抑制细胞因子的不良作用^[8];黄芪具有调节免疫^[9],改善血液流变学作用;冬虫夏草具有调节机体免疫力^[10],促进I、Ⅲ型胶原降解的作用^[11];当归具有抗炎、抗自由基作用^[12];三七具有抗炎、调节机体免疫力的作用^[13];板蓝根具有抗病毒、抗菌等作用^[14]。肝纤维化和肾纤维化基本病理改变是相同的,即纤维生成细胞分泌器官纤维化因子增加,诱发细胞外基质合成增多与降解减少,纤维结缔组织增生,最终导致器官纤维化。因此,可以采用相同的治则及方药进行治疗。

UUO 是一种经典的肾间质纤维化模型,本实验在术后第4周模型组大鼠左侧肾脏明显增大,皮质明显变薄,呈囊状,颜色暗红,表面粗糙不平,有囊肿和瘢痕。镜下可见模型组肾小管结构已严重破坏,管腔塌陷,上皮细胞大量坏死;有些部位肾小管和间质的结构基本消失,间质中出现弥漫性细胞增殖;有些部位呈现广泛的纤维化。胶原染色显示间质中弥漫纤维增生、积聚,其中可见大量I型和Ⅲ型胶原沉积。六胺银染色显示肾小管基底膜增粗,有的内折。这和以往的文献报道是一致的^[15-16]。模型组Scr、BUN及尿NAG酶和 β_2 微球蛋白均明显高于正常组,但并没有超出正常范围,初步认为这是由对侧肾脏代偿的结果。术后4周从病理上表现为肾间质纤维化,从以上结果均证实该模型具有典型的肾间质

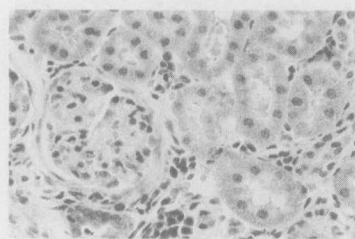


图7 复方鳖甲软肝方中剂量组大鼠肾组织,正常肾小球、肾小管,间质有轻度炎细胞浸润,HE×400

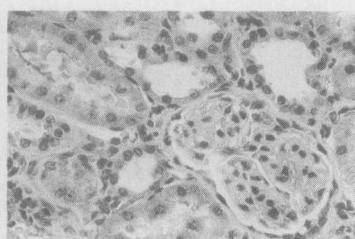


图8 复方鳖甲软肝方大剂量组大鼠肾组织,肾小管扩张,上皮细胞脱落,上皮细胞空泡样变;轻度炎细胞浸润,轻度纤维化;球囊壁轻度粘连,轻度系膜细胞增生,HE×400

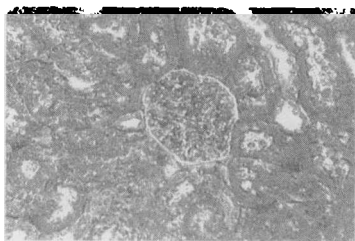


图9 正常组大鼠肾组织,正常结构肾小球、肾小管, Mallory×200

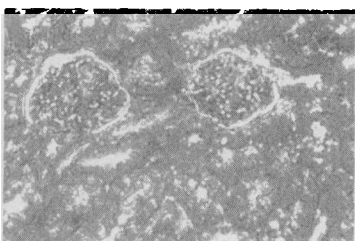


图10 假手术组大鼠肾组织,正常结构肾小球、肾小管 Mallory×200

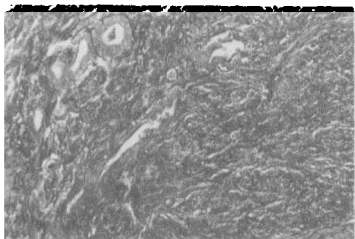


图11 模型组大鼠肾组织,肾小管间质纤维化,间质中大量胶原纤维, Mallory×200

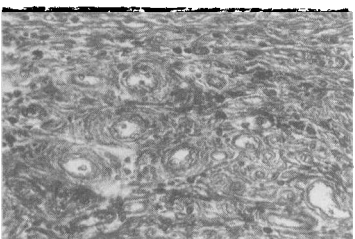


图12 模型组大鼠肾组织,肾小管被大量胶原纤维代替, Mallory×200



图 13 苯那普利组大鼠肾组织, 肾小管间质轻度纤维化, 肾小管萎缩, Mallory×200

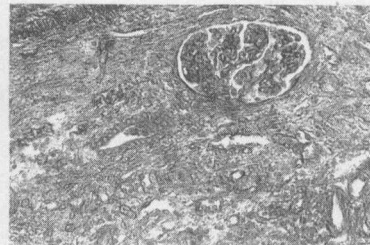


图 14 复方鳖甲软肝方小剂量组大鼠肾组织, 肾小管间质中度纤维化, 肾小管萎缩, 结构不清, Mallory×200

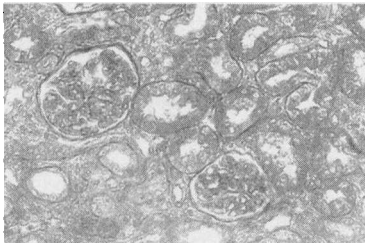


图 15 复方鳖甲软肝方中剂量组大鼠肾组织, 肾小球、肾小管结构基本正常, 有少量胶原纤维沉积, Mallory×200

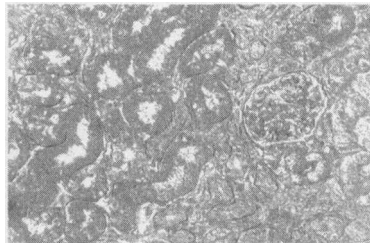


图 16 复方鳖甲软肝方大剂量组大鼠肾组织, 肾小球肾小管结构基本正常, 有少量胶原纤维沉积, Mallory×200

纤维化病理特征。

应用 FFBJRGF 治疗的大鼠左侧肾脏也有所增大, 但肾重体重比与模型组比较有所减轻, 解剖可见囊肿、瘢痕明显减少; 显微镜下观察病变明显减轻, 纤维化病变呈灶性, 积聚的胶原成分减少, 肾小管结构较完整, 基底膜增粗、内折现象减少。本实验镜下观察各组肾组织病理切片发现, 在结扎输尿管的大鼠肾间质中有大量炎细胞浸润, 而治疗组炎细胞有不同程度的减少。大鼠肾组织病理学分析结果表明 FFBJRGF 高、中、低剂量组肾间质纤维化的积分值明显低于模型组大鼠。在功能上, FFBJRGF 治疗的大鼠 Scr、BUN 及尿 NAG 酶和 β_2 微球蛋白均明显低于模型组。从上述结果可以看出, FFBJRGF 可延缓肾间质纤维化的发展进程, 其中 FFBJRGF 中剂量组效果较好, 这可能是因为高剂量药物灌胃时吸收度不好而不能发挥较好的疗效。复方鳖甲软肝方可延缓单侧输尿管结扎肾间质纤维化的病程进展, 但其确切机理是如何通过减少炎细胞的浸润、减轻肾小管的损伤, 进而延缓肾小管萎缩、肾间质纤维化的进程有待

进一步实验观察和探讨。

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Objective To study and make a new device to be used for the acquisition of pulse message in traditional Chinese medicine, whose feeler is coupled with an ultrasonic apparatus and a pressure sensor, including photoelectric volume sensors and electrocardio-sensors and to acquire pulse condition after processing collected multi-divisional signals by computer in order to facilitate objective diagnosis. **Method** to conduct iconographic analysis of the data of the movement of radial artery collected by the device. **Result** It is proved that the reliability of the device is quite high by applying instruments to detect the ultrasonogram of normal Cunkou radial artery, measuring the distance between apro-capitulum radius and akin and testing the change of inradius and vasomotion, and then to compare it with its Doppler image and that its stability is satisfactory via image analysis in different time intervals. **Conclusion** This new device for the acquisition of pulse message in TCM is simple in operation, able to supply a plenty of messages and its reliability and stability are appreciable, by which the movement of cunkou radial artery can be viewed by the way of direct and real-time observation.

Key words pulse-taking apparatus, B- ultrasound, pressure

Experimental Studies on Action Mechanisms of Compound Prescription Biejiarangan in Prevention and Treatment of Interrenal Fibrosis of Rats

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Objective To investigate the action of the compound prescription Biejiarangan on the blood biochemistry and the pathomorphology of renal tissues of rats with interrenal fibrosis by the way of an unilateral ureteral obstruction (UUO) model. **Method** To divide the rats for experiments into the following seven groups: normal, sham operation, model, Benazepril treatment and three groups of rats fed with low, medium and high dosages of the compound prescription Biejiarangan respectively, and to kill the rats in four weeks after UUO operation and to dye with HE, PAM and Mallory in order to observe the pathological change of the renal tissues of the rats. **Result** In the model group the structure of all the kidney tubules of the rats is heavily damaged with their lumina collapsed and their epithelial cells are largely in necrosis. In some parts the structure of the kidney tubules and the mesenthyne of the rats basically disappear and cell proliferation emerges in the mesenthyne in a diffuse way and the fibrosis of the mesenthyne gets widespread. In the groups of the rats fed with low, medium and high dosages of the compound prescription Biejiarangan and in the group of Benazepril treatment the pathological change of interrenal fibrosis of the rats becomes apparently mitigated and it is particularly evident in the group of the rats fed with medium dosages. **Conclusion** The compound prescription Biejiarangan is able to reduce the infiltration of inflammatory cells, lessen the damage of kidney tubules and then retard the progress of interrenal fibrosis.

Key words unilateral ureteral obstruction (UUO), compound prescription Biejiarangan, interrenal fibrosis,

action mechanism

Present situation of study on 5-hydroxymethyl-2-furfural

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This article summarizes the general process of the formation of 5-HMF compound and the status quo of its existence, sums up its pharmacological action and briefly describes the present situation of the study of 5-HMF in compound prescriptions of traditional Chinese medicine. And on this basis the authors of the article hold that 5-HMF may be an unknown active component and can produce original effect in TCM compound prescriptions.

Key words 5-hydroxymethyl-2-furfural, traditional Chinese medicine

Exploration of Factors Exerting Influence on Pharmacological Action and Clinical Effect of Prescriptions of Traditional Chinese Medicine

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Based on medicinal materials, human body and environment this article describes the factors which are able to exert influence on the pharmacological action and clinical effect of the prescriptions of traditional Chinese medicine, thus providing pharmacological study with more references. It tells people to assess the role of TCM prescriptions in a scientific way so as to give a better play to their effect of treatment.

Key Words Prescription, pharmacological action, clinical effects, influencing factors

Construction of component library of kudzu root

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In this study TCM components are taken for its model, the construction and development of TCM component library are regarded as its core and the root of kudzu is chosen as its object to explore the method for the separation and characterization of TCM components. As a result a method for efficient preparation and separation of scale components has been developed and 18 components from n-butyl alcohol fraction have been obtained due to the application of industrial chromatograph and other related techniques, and 18 fingerprints of component chemistry have been achieved by HPLC, which have been preliminarily characterized via comparison of them with standard ones.

Key words component library, kudzu root, industrial chromatograph, HPLC