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广枣叶总黄酮对心肌缺血再灌注损伤模型大鼠的保护作用及其作用机制

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摘要: **目的** 分析广枣叶总黄酮灌胃对心肌缺血再灌注损伤大鼠心肌功能的保护作用,并探讨可能存在的作用机制。**方法** 将60只健康SPF级大鼠作为研究对象,采用随机数字表法将大鼠分为5组,分别为伪手术组、阳性对照组、模型组、小剂量给药组、大剂量给药组。阳性对照组给予0.02 g·kg^{-1}维拉帕米灌胃,小剂量组给予0.2 g·kg^{-1}广枣叶总黄酮混悬液灌胃,大剂量组给予0.4 g·kg^{-1}广枣叶总黄酮混悬液灌胃,伪手术组与模型组均给予同等容量生理盐水灌胃。用药7 d后,除伪手术组大鼠,其余4组大鼠均制备心肌缺血再灌注损伤模型。手术完毕将5组大鼠处死,分别进行血清生化、心肌生化、心律失常、心肌梗死和组织形态学等指标检测与比较,重点探讨广枣叶总黄酮对大鼠再灌注损伤的保护效果与作用机制。**结果** 与伪手术组相比,模型组大鼠的SOD活性降低,MDA、CK、LDH含量明显升高,室性心动过速、室颤发生次数及持续时间升高,出现严重心肌梗死,各指标比较差异均有统计学意义($P<0.05$)。阳性对照组、小剂量给药组与大剂量给药组在用药7 d后,上述各指标均得到不同程度改善,相较模型组差异显著($P<0.05$)。伪手术组大鼠心肌胶原纤维整齐排列,形态正常,无红细胞及炎细胞浸润改变,而模型组心肌原纤维增多,排列紊乱,细胞核固缩,其余3组心肌组织病理改变得到修复,其中大剂量给药组心肌组织形态改善更显著。**结论** 广枣叶总黄酮对心肌缺血再灌注损伤大鼠具有保护作用,其可能通过抑制丙二醛生成,减少氧自由基,降低心肌酶漏出等机制,控制炎症反应、改善心肌梗死的不良后果。

关键词: 心肌缺血再灌注损伤; 大鼠模型; 广枣叶总黄酮; 心肌保护; 作用机制
中图分类号: R 96 **文献标志码:** A

心肌缺血后再灌注损伤是指患者发生心肌缺血后一定时间内获得血液灌注,但恢复正常灌注的心肌组织损伤却出现进行性加重的一种病理过程[1-2]。心肌缺血后再灌注损伤在临床上比较常见,对患者生存质量造成了严重威胁,虽然临床对该病高度重视,并通过多种手段加以干预治疗,但至今尚无一种药物能有效、彻底修复心肌组织坏死面积[3-4]。

目前,医学界应用不同药物治疗心肌缺血后再灌注后损伤大鼠的实验诸多,都旨在寻求一种能显著保护心肌功能的高效药物[5-6]。多项研究指出,黄酮类化合物具有显著的抗感染、抗氧化、改善血液循环等作用,对心肌缺血再灌注心脏具有较好的保护效果,有助于心肌缺血再灌注后损伤的治疗[7-8]。本文作者以心肌缺血再灌注损伤大鼠模型为实验对象,分析了广枣叶总黄酮对大鼠心肌的保护作用,并探讨其作用机制,为心肌缺血再灌注损伤的有效治疗提供依据。

1 仪器与材料

SA430型小动物人工呼吸机(江苏赛昂斯生物科技有限公司),Olympus BX63型荧光自动显微镜(日本Olympus株式会社),ECG-2150型心电图机(上海光电医用电子仪器有限公司),CP323C型电子天平(奥豪斯仪器上海有限公司),Calibur流式细胞仪(美国B&D公司),420S型生物信号采集与处理系统(成都泰盟科技有限公司)。

广枣叶总黄酮(广东海洋大学理学院提供,批号:20070610,提取物中总黄酮类化合物含量质量分数≥5%,根据实验要求加蒸馏水配制成相应浓度的总黄酮混悬液),维拉帕米(广东华南药业

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集团有限公司，批号：国药准字 C14200003497），丙二醛（malondialdehyde，MDA）、超氧化物歧化酶（superoxide Dismutase，SOD）、肌酸激酶（creatine kinase，CK）等试剂盒（南京卡米洛生物工程有限公司），乳酸脱氢酶（lactate dehydrogenase，LDH）试剂盒（中生北控生物科技股份有限公司）。

健康 SPF 级大鼠 60 只，8～10 周，由上海斯莱克实验动物有限公司所提供，动物生产许可证号：SCXK（沪）2017－0005，质量合格证号：2017000560136；所选大鼠雌雄性各占一半，平均体质量：（250±25）g。

2 方法与结果

2.1 分组方法及实验

开始正式实验前先另取大鼠进行预实验，以确定大鼠经口染毒的剂量范围。根据预实验结果，采用随机数字表法将大鼠分为 5 组，每组 12 只，分别为伪手术组、阳性对照组、模型组、小剂量给药组、大剂量给药组。

大鼠均在恒温恒湿的实验条件下喂养 3 d，在给药前 12 h 禁食（不禁水）。其中，阳性对照组予以 0.02 g·kg^{-1}维拉帕米灌胃；小剂量给药组予以 0.2 g·kg^{-1}广枣叶总黄酮混悬液灌胃；大剂量给药组予以0.4 g·kg^{-1}广枣叶总黄酮混悬液灌胃；伪手术组与模型组给予同等容量的生理盐水灌胃。5 组大鼠均是每天用药 1 次，连续用药 7 d。给药后观察大鼠的反应，采用常规法饲养，观察大鼠行为活动、死亡等情况。

用药 1 周后，除伪手术组大鼠，其余各组大鼠均制备心肌缺血再灌注损伤模型。大鼠取仰卧位固定，应用质量分数为 10 % 的乌拉坦，按照 10 mL·kg^{-1}用药剂量进行麻醉，标准步骤开胸后，连接小动物人工呼吸机，设置潮气量为 6～8 mL，呼吸频率为 70～80 次/min，应用针型电极记录标准肢体的Ⅱ导联心电图。在大鼠胸骨左缘 3、4 肋间切开胸壁，充分暴露出心脏，对左冠状动脉前降支进行结扎，结扎线由直径 2 mm 的 PE 管中穿出。PE 管的一端用来阻断冠状动脉血流，另一端采用动脉夹进行固定。致缺血 30 min 后松开结扎线，取出 PE 管，再进行 2 h 的灌注。对于伪手术组，进行前室间支分离，不予结扎。

2.2 生化指标及心肌功能指标检测

手术完毕后，处死大鼠，取右心房静脉血 5 mL，2 000 r·min^{-1}离心 10 min，吸取上清液进行血清生化指标 LDH、CK 含量测定。同时迅速取出大鼠心脏，放入生理盐水里冲洗后置入－80 ℃冰箱待用。将心脏冷冻 20～30 min 后，精密称取 0.5～1 g，以体积分数为 0.86 % 的生理盐水作为匀浆介质，加入心肌组织于 4 ℃下制备成体积分数为 10 % 的组织匀浆。3 000 r·min^{-1}离心 15 min，吸取上清液进行 MDA 含量、SOD 活性测定。

经由生物信号采集与处理系统记录大鼠的Ⅱ导心电图，对大鼠再灌注期出现的室颤、室性心动过速的发生次数、持续时间等指标进行记录。

将大鼠心脏自结扎线以下横向均匀切片，切厚度相等的 5 片，称质量，N－BT 染色，应用多媒体病理分析系统对切片进行病理分析，固定象距下测量梗塞心肌与正常心肌的面积，并对心肌梗死区的重量、梗死区占心室主心脏的比重进行计算。

2.3 统计学处理

数据应用 SPSS 18.0 软件行统计处理，计量资料以（$\bar{x}\pm s$）描述，比较时对数据行方差齐性检验，方差齐则行 t 检验或单因素方差分析；方差不齐则行秩和检验。统计学差异用 $P<0.05$ 表示。

2.4 各组生化指标检测分析

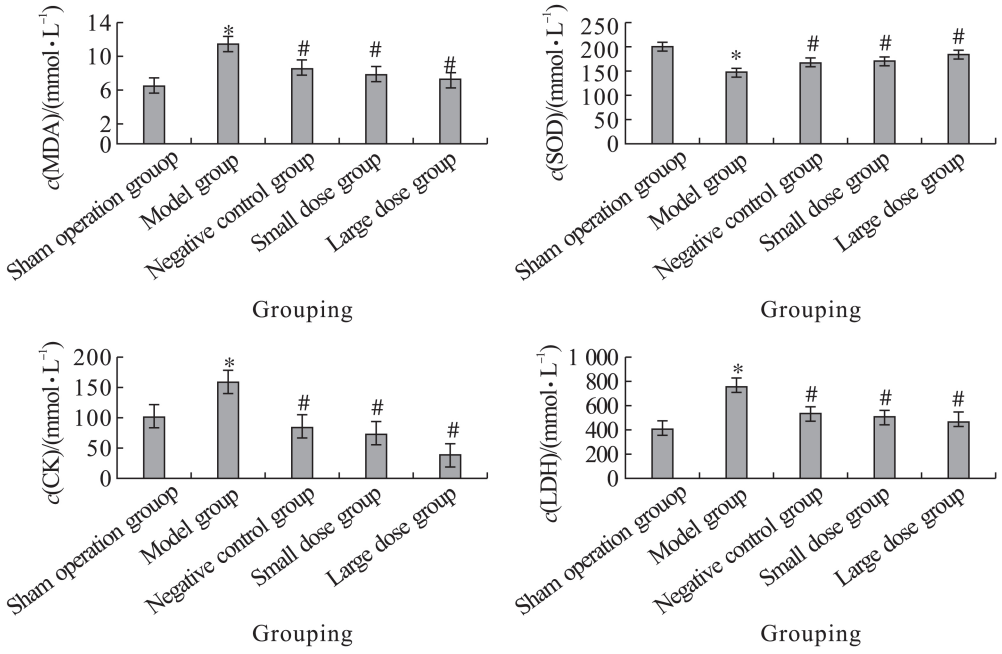
相较于伪手术组，模型组大鼠的 MDA 含量升高，SOD 活性降低，CK、LDH 含量均明显升高（$P<0.05$）；相较于模型组，阳性对照组与大、小剂量广枣叶总黄酮给药组 MDA、CK、LDH 含量呈逐组降低趋势，SOD 活性呈逐组升高趋势（$P<0.05$）。见图 1 所示。

2.5 各组心电图监测指标分析

伪手术组大鼠无室性心动过速、室颤发生，而模型组室性心动过速、室颤发生次数及持续时间均高于阴性对照组、小剂量与大剂量广枣叶总黄酮给药组，差异比较有统计学意义（$P<0.05$），结果见表 1。

2.6 各组心肌梗死指标分析

伪手术组大鼠无梗死，模型组大鼠有严重梗死。相较于模型组，维拉帕米用药的阳性对照组显著降低了心肌缺血再灌注导致的心肌梗死（$P<0.05$）。且大剂量广枣叶总黄酮给药组梗死心肌面积、梗死区重量、梗死区占心室、心脏比重更低于阳性对照组（见表 2）。



* —$P<0.05$ compared with sham operation group; #—$P<0.05$ compared with model group

Fig. 1　Detection and analysis of biochemical indicators in each group

图 1　各组生化指标检测分析

Table 1　Number and duration of ventricular tachycardia and ventricular fibrillation in rats

表 1　大鼠室性心动过速、室颤发生次数及持续时间

Group	Quantity (only)	Ventricular tachycardia		Ventricular fibrillation	
		$t_{occurrence}$/s	$t_{duration}$/min	$t_{occurrence}$/s	$t_{duration}$/min
Sham operation	12	0	0	0	0
Model	12	8.4 ± 1.28	23.6 ± 8.72	7.5 ± 1.46	21.5 ± 8.20
Negative control	12	5.8 ± 1.20	20.8 ± 6.54	4.0 ± 1.46	18.8 ± 8.42
Small dose	12	5.1 ± 1.05	18.2 ± 5.18	3.8 ± 1.22	17.8 ± 5.84
Large dose	12	4.6 ± 0.72	17.0 ± 6.04	3.3 ± 1.38	16.9 ± 5.42

Table 2　Analysis of myocardial infarction indicators in each group

表 2　各组心肌梗死指标分析

Group	Quantity (only)	Infarcted myocardial area/%	$m_{infarct}$/g	Infarct/ventricular/%	Infarcted area/heart/%
Sham operation	12	0	0	0	0
Model	12	46.2 ± 8.54*	0.083 ± 0.015*	16.8 ± 3.72*	12.0 ± 2.18*
Negative control	12	35.4 ± 9.27#	0.065 ± 0.012#	11.5 ± 2.84#	8.8 ± 1.75#
Small dose	12	33.4 ± 9.13#	0.061 ± 0.014#	11.2 ± 2.67#	8.3 ± 2.08#
Large dose	12	31.7 ± 8.50#	0.051 ± 0.009#	10.8 ± 1.96#	7.2 ± 2.04#

* —$P<0.05$ compared with sham operation group; #—$P<0.05$ compared with model group

2.7　各组心肌组织形态学指标分析　未用药、未造模的伪手术组大鼠心肌胶原纤维整齐排列，结构完整，形态正常，未见细胞水肿、红细胞及炎症细胞浸润等病理改变; 而模型组大

鼠心肌原纤维增多，排列紊乱，细胞断裂、肿胀，细胞核固缩；维拉帕米用药的阳性对照组大鼠较模型组心肌细胞形态有所改善，但仍有核固缩现象，心肌纤维溶解断裂，间质充血，炎细胞浸润等改变；小剂量广枣叶总黄酮给药组心肌胶原纤维排列较为紊乱，存在炎细胞、红细胞浸润现象；大剂量广枣叶总黄酮给药心肌胶原纤维排列相对整齐，纤维呈局域性紊乱排列，间质中炎细胞与红细胞浸润改善。见图2所示。

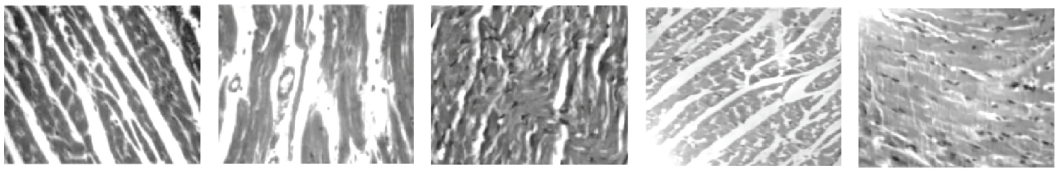


Fig. 2 Analysis of myocardial tissue morphology in each group (HE staining, ×400)

图2 各组心肌组织形态学指标分析(HE染色，×400)

3 讨论与结论

心肌缺血再灌注损伤在临床十分常见，其会使心脏出现比之前更为严重的结构损伤及代谢功能障碍。目前认为，黄酮类化合物能够起到抗心肌缺血，改善动脉粥样硬化的作用，用于心肌缺血再灌注的治疗比较可靠[9-10]。黄酮类化合物在植物界中广泛存在，在广枣中就含有丰富的总黄酮类化合物，广枣的抗炎、抗氧化、消除自由基、改善心肌缺血等药理作用已得到临床一致肯定。但以往临床主要通过干燥成熟的广枣果实来提取总黄酮类化合物，将其用于心肌缺血、心肌梗死等疾病的治疗，对于广枣叶都是弃之不用的。近年来有研究认为，相较于取材季节受限、成本较高的广枣果实，同样含有总黄酮类化合物的广枣叶无疑是更低价、产量更充足、毒性更低的替代品[11-12]。

本文作者以心肌缺血再灌注损伤模型大鼠为实验对象，主要分析了广枣叶总黄酮用于大鼠模型后对心脏的保护作用及作用机制。为了解广枣叶总黄酮用于大鼠心肌的最大无毒浓度，正式实验前先经过预实验进行了最大给药量测定，了解到大鼠的广枣叶总黄酮最大给药量约为40 $g \cdot kg^{-1}$，提示本实验中的小剂量(0.2 $g \cdot kg^{-1}$)、大剂量(0.4 $g \cdot kg^{-1}$)广枣叶总黄酮灌胃基本无毒性，属于比较安全的实验用药剂量。

心肌缺血大鼠发生再灌注损伤后，会产生大量的自由基，同时会使SOD活性受到明显抑制，从而无法及时、有效清除自由基。自由基作用于心肌细胞膜后，与生物大分子及细胞膜发生过氧化反应，产生大量的MDA，对心肌细胞造成损伤，细胞内CK、LDH含量水平降低[13-14]。在本实验中，模型组大鼠较伪手术组大鼠便出现了显著的SOD活性降低，MDA、CK、LDH水平升高的现象。而应用维拉帕米的阳性对照组，SOD活性得到增加，MDA水平降低，CK、LDH漏出率得到改善，与模型组相比差异明显($P<0.05$)。同样的，大剂量与小剂量广枣总黄酮给药组各SOD、MDA、CK、LDH指标也有改善，且改善水平更优于阳性对照组，可见总黄酮用药对改善大鼠心肌缺血再灌注损伤有显著效果。

分析各组的心电图监测指标与心肌梗死情况发现，大鼠经心肌缺血再灌注造模后，心动过速、心颤发生率明显升高，且持续时间长，梗死心肌面积、重量、梗死区占心室比重也显著增加，未造模的伪手术组并无上述现象。而对造模大鼠用药干预后，不论是维拉帕米用药的对照组，还是广枣叶总黄酮大、小剂量给药组，上述指标均得到显著改善。提示广枣叶总黄酮类化合物与西药维拉帕米一样，均能起到扩张血管、增加缺血心肌冠脉流量、抗心律失常的作用。在对各组大鼠的心肌组织形态学分析中也发现，经再灌注建模后，大鼠心肌原纤维增多，排列紊乱，细胞断裂、肿胀，细胞核固缩，但广枣叶总黄酮用药组与阳性对照组的心肌组织损伤程度明显轻于模型组。结合实验结果认为，广枣叶总黄酮对心肌缺血再灌注损伤大鼠心肌保护的作用机制可能如下：广枣叶总黄酮可通过减少氧自由基产生，抑制心肌脂质过氧化，纠正心肌炎性反应，降低心脑组织中MDA等含量，对缺血再灌注损伤的心肌起到保护作用，这一机制与其他植物中总黄酮的作用机制基本符合[15-16]。本次研究结果进一步显示了广枣叶总黄酮类化合物用于心肌缺血再灌注损伤的可行性

与合理性。

广枣叶总黄酮具有明显的抗心律失常、抗氧化、保护心肌缺血等作用,对改善心肌缺血再灌注损伤的疗效显著。

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Myocardial protective function and mechanism of total flavonoids from Jujube leaves after myocardial ischemia – reperfusion injury in rats

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Abstract: Objective To analyze the protective effect of total flavonoids from jujube leaves on myocardial function in rats after myocardial ischemia-reperfusion injury,and to explore the possible mechanism of action. **Methods** Sixty healthy SPF rats were used as the study subjects. The rats were divided into five groups by random number table method,which were sham operation group,positive control group,model group,low dose

group and high dose group. The positive control group was given 0.02 g·kg^{-1} verapamil orally; the low dose group was given 0.2 g·kg^{-1} Guangzao leaf total flavonoid suspension; and the high dose group was given 0.4 g·kg^{-1} Guangzao leaf total flavonoid suspension by intragastric administration; the sham operation group and the model group were given the same volume of normal saline. After 7 days of treatment, except for the rats in the sham operation group, the other four groups of rats were prepared for myocardial ischemia-reperfusion injury model. Five groups of rats were sacrificed after operation, and serum biochemistry, myocardial biochemistry, arrhythmia, myocardial infarction and other indicators were detected and compared, and the protective effect and mechanism of total flavonoids from jujube leaves on reperfusion injury in rats were studied. **Results** Compared with the sham operation group, the SOD activity of the model group decreased, the MDA, CK, LDH levels increased significantly, the ventricular tachycardia, the number and duration of ventricular fibrillation increased, and severe myocardial infarction occurred, and the differences between the indicators were statistically significant ($P < 0.05$). The positive control group, the low-dose group and the high-dose group were all improved in different degrees after 7 days of treatment, which was significantly different from the model group ($P < 0.05$). In the sham operation group, the myocardial collagen fibers were neatly arranged, the morphology was normal, and no red blood cells and inflammatory cells infiltrated, while the model group had increased myocardial fibrils, disordered arrangement, nucleus pyknosis, and the remaining three groups of myocardial tissue pathological changes were repaired, of which large doses were obtained, among them the myocardial tissue morphology of the high-dose group was improved more significantly. **Conclusion** The total flavonoids from jujube leaves have protective effects on myocardial ischemia-reperfusion injury in rats, which may be through inhibit malondialdehyde production, reduce oxygen free radicals, reduce myocardial enzymes and other mechanisms, to control the inflammatory response and improve myocardial infarction.

Key words: myocardial ischemia-reperfusion injury; rat model; total flavonoids from leaves of jujube; myocardial protection; mechanism of action

(上接第1002页)

Determination of four residual solvents in celecoxib raw material by gas chromatography with head-space sampling

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Abstract: Objective To establish a method for the determination of 4 kinds of organic residual solvents in celecoxib raw material. **Methods** The samples were separated on a DB-624 capillary column (30 m × 0.53 mm, 3.0 μm) using the temperature programming and analyzed with a FID detector. The temperature of injector port and the flame ionization detector was 250 ℃. Nitrogen was used as the carrier gas with a flow rate of 2.0 mL·min^{-1}. The spilt ratio was 5∶1. The head-space equilibrium temperature was set at 80 ℃, and the equilibrium time was 30 min. **Results** The linear ranges of methanol, ethanol, ethyl trifluoroacetate and ethyl acetate were well separated, and a good linearity was obtained within the designed range for each solvent (r = 0.998 9 – 0.999 6). The average recoveries of four residual solvents were in the range of 91.96% to 111.18%. **Conclusion** The method is rapid, accurate and sensitive for the content determination of residual solvents in celecoxib raw material.

Key words: head-space capillary gas chromatography; celecoxib; residual solvents