蕨麻正丁醇提取部位对小鼠急性心肌缺血损伤的保护作用

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目的：研究蕨麻正丁醇提取部位（n-butanol extract of Potentilla anserina L.，NP）对垂体后叶素致小鼠急性心肌缺血损伤的保护作用。

方法：90 只雌性昆明种小鼠随机分为正常对照组、缺血模型组、复方丹参组和 NP 低、中、高剂量组，每组 15 只。除正常对照组外，各组小鼠通过腹腔注射垂体后叶素（20 U/kg）建立急性心肌缺血模型。检测模型后心电图示 J 波上移变化，测定小鼠血清乳酸脱氢酶（lactate dehydrogenase，LDH）、肌酸激酶（creatine kinase，CK）、超氧化物歧化酶（superoxide dismutase，SOD）活性及丙二醛（malondialdehyde，MDA）含量，并通过 Nagar-Olsen 染色观察小鼠心肌缺血损伤程度。

结果：与缺血模型组比较，各治疗组缺血所致心电图 J 波上的上移明显显著降低（P < 0.01）。中、高剂量 NP 及复方丹参组显著降低缺血损伤小鼠血清中 LDH、CK 活性（P < 0.01，P < 0.05），提高小鼠血清中 SOD 活性（P < 0.01），减少 MDA 的产生（P < 0.05），而低剂量 NP 组小鼠血清中 LDH、CK、SOD 活性及 MDA 含量，与模型组比较差异无统计学意义（P > 0.05）。Nagar-Olsen 染色显示，中、高剂量 NP 及复方丹参组显著减少小鼠心肌缺血损伤红染面积，而低剂量 NP 组仍可见大片红染的损伤心肌。

结论：蕨麻正丁醇提取部位对急性心肌缺血损伤具有保护作用。

关键词：蕨麻；心肌缺血；超氧化物歧化酶；肌酸激酶；乳酸脱氢酶；丙二醛；小鼠

Protective effects of n-butanol extract of Potentilla anserina on acute myocardial ischemic injury in mice

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Objective: To investigate the protective effects of the n-butanol extract of Potentilla anserina L. (NP) on pituitrin-induced acute myocardial ischemic injury in mice.

Methods: Ninety healthy female mice were randomly divided into normal control group, untreated group, Salvia miltiorrhiza group and low-, medium- and high-dose NP groups. Except for the normal control group, the mice were intraperitoneally injected with pituitrin (20 U/kg) to induce acute myocardial ischemic injury. Thirty minutes after induction, electrocardiogram was monitored, and height of the J spot was measured also. Activities of lactate dehydrogenase (LDH), creatine kinase (CK) and superoxide dismutase (SOD) and content of malondialdehyde (MDA) in serum of the mice were detected. The degree of myocardial ischemic
injury in mice was observed by Nagar-Olsen staining.

**Results:** The moving up of J spots in the treated groups was significantly inhibited when comparing with the untreated group ($P < 0.01$). Compared with untreated group, high- and medium-dose NP and *Salvia miltiorrhiza* could significantly decrease the activities of LDH, CK ($P < 0.01, P < 0.05$), increase the SOD activity ($P < 0.01$) and decrease the content of MDA ($P < 0.05$). However, no significant difference was observed between low-dose NP group and untreated group ($P > 0.05$). Nagar-Olsen staining showed that high- and medium-dose NP and *Salvia miltiorrhiza* could significantly diminish the areas of cardiac muscles injured by ischemia, but low-dose NP had no effect on that.

**Conclusion:** NP has a remarkable protective effect on acute myocardial ischemic injury in mice.

**Keywords:** *Potentilla anserina* L.; myocardial ischemia; superoxide dismutase; creatine kinase; lactate dehydrogenase; malonaldehyde; mice

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1. Materials and Methods

1.1 **Drug and Chemicals.** Aconite, *Salvia miltiorrhiza* L., *Potentilla anserina* L., and *Rehmannia officinalis* were obtained from commercial sources. All other chemicals were of analytical grade.

1.2 **Animals.** Male Sprague-Dawley rats (250-300 g) were obtained from the Laboratory Animal Center, Shanghai, China. The animals were housed in a temperature-controlled environment with a 12-hour light/dark cycle.

1.3 **Experimental Protocol.** A total of 120 rats were randomly divided into 4 groups: control, ischemia, ischemia + reperfusion, and ischemia + reperfusion + NP (30 mg/kg). NP was administered intraperitoneally 30 min before reperfusion. The rats were sacrificed at 24 h after reperfusion, and the hearts were collected for analysis.

1.4 **Histological Analysis.** hearts were fixed in 10% formalin, embedded in paraffin, and sectioned at 5 μm. The sections were stained with hematoxylin and eosin (H&E) for histological analysis.

1.5 **Statistical Analysis.** All data were expressed as mean ± SD. The statistical differences were analyzed using one-way ANOVA followed by Dunnett's test. *P* < 0.05 was considered statistically significant.

2. Results

2.1 **Effect of NP on Myocardial Infarct Size.** The results showed that NP significantly reduced the infarct size in the ischemia + reperfusion + NP group compared to the ischemia + reperfusion group.

2.2 **Histological Findings.** The H&E-stained sections showed that NP treatment significantly reduced the extent of myocardial necrosis and improved the myocardial architecture.

3. Discussion

3.1 **Roles of NP.** NP has been shown to have a protective effect on myocardial ischemia-reperfusion injury. The mechanisms include antioxidant, anti-inflammatory, and anti-apoptotic effects.

3.2 **Future Directions.** Further studies are needed to elucidate the underlying mechanisms of NP's protective effects and to explore its potential clinical applications.

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4. Conclusion

NP effectively protected against myocardial ischemia-reperfusion injury, probably through its antioxidant and anti-inflammatory properties. Further studies are needed to confirm these findings and to explore the clinical potential of NP.
2.2 小鼠血清 LDH 和 CK 活性 与正常对照组比较，模型组小鼠血清 LDH 和 CK 活性显著升高（P<0.01），各治疗组血清 LDH 和 CK 活性亦明显升高（P<0.01, P<0.05）。与模型组相比，复方丹参及高、中剂量 NP 组血清 LDH 及 CK 活性均显著降低（P<0.01, P<0.05）；低剂量 NP 组血清 LDH, CK 活性与模型组比较差异无统计学意义（P>0.05）。见表 2。

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LDH</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>15</td>
<td>1651±575</td>
<td>336±193</td>
</tr>
<tr>
<td>Untreated</td>
<td>15</td>
<td>5769±827</td>
<td>255±193</td>
</tr>
<tr>
<td><em>Salvia miltiorrhiza</em></td>
<td>15</td>
<td>4277±878</td>
<td>1304±360*</td>
</tr>
<tr>
<td>Low-dose NP</td>
<td>15</td>
<td>5948±786</td>
<td>2880±712*</td>
</tr>
<tr>
<td>Medium-dose NP</td>
<td>15</td>
<td>4752±766*</td>
<td>382±360*</td>
</tr>
<tr>
<td>High-dose NP</td>
<td>15</td>
<td>2650±917*</td>
<td>715±340*</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01, vs normal control group; △△ P<0.05, △△△ P<0.01, vs untreated group.

2.4 心肌 Nagar-Olsen 染色 光学显微镜下可见，正常对照组心肌组织呈黄染，模型组心肌呈红染。与模型组比较，高、中剂量 NP 及复方丹参组心肌红染面积显著减少，黄染面积增多；而低剂量 NP 组虽有部分组织黄染，仍可见大片红染的心肌组织。图见图 1.
图 1 各组小鼠心肌组织 Nissl-Ofen 染色（光学显微镜，×200）

Figure 1 Nissl-Ofen staining of cardiac muscles of mice in different groups (Light microscopy, ×200)

A. Normal control group; B. Untreated group; C. Saline wet control group; D. Low-dose NP group; E. Medium-dose NP group; F. High-dose NP group.

3 讨论

垂体后叶素是从动物脑垂体后叶提取的一种水溶性物质，能够收缩血管特别是毛细血管和小动脉，导致血压升高、心肌缺血缺氧。近年来，利用垂体后叶素制备心肌缺血动物模型已应用于筛选抗心肌缺血药物的研究。本实验应用垂体后叶素腹腔注射建立小鼠急性心肌缺血模型，通过检测心电信号变化、血清肌酸激酶变化及心肌组织病理学染色，观察脱敏有效部位对小鼠急性心肌缺血损伤的影响。

心电图变化是心肌缺血模型成功与否的最直观的指标。QRS 波群的终点与 ST 段转换处称为 J 点，J 点上移，ST 段抬高或降低 0.1 mV 以上，T 波高耸且波幅均显著为心肌缺血的心电图表现。通常大鼠、家兔、狗等动物心肌缺血模型的心电图表现可由 ST 段的变化显示，但由于小鼠心电图 ST 段不显著，故可将 J 点变化作为判定模型成功与否的观察指标 [3]。本实验结果显示，小鼠腹腔注射垂体后叶素后心电图 J 点明显上移，提示心肌缺血模型复位成功，并且所有心电图参数显著降低的电位与 J 点的变化一致，进一步验证模型成功；各剂量垂体后叶素提取物对小鼠心肌缺血性心电图的影响，提示其对急性心肌缺血损伤有一定的保护作用。

LDH 是参与细胞能量代谢的一个重要的酶，可以催化乳酸生成丙酮酸，同时产生三磷酸腺苷。心肌缺血损伤时，细胞膜完整性破坏，通透性增加，LDH 将从细胞中释放出来，因此 LDH 是反映细胞膜完整性的重要检测指标 [32]。CK 也是重要的心肌酶之一，其释放量直接反映了心肌缺血损伤的程度。

血清中 LDH、CK 活性越高，表明心肌损伤越严重。本实验结果显示，高、中剂量薰衣草全酶提取物显著降低小鼠心肌 LDH、CK 活性，进一步提示其抗心肌缺血作用。

常规的 HE 染色很难分辨心肌缺血的早期改变，但通过观察 HE 染色才能显示心肌组织的病理变化。Nissl-Ofen 染色的原理为：缺血心肌对复红液的着色力较强，分化时不脱落色。缺血性损伤的心肌组织呈红色，而正常心肌对分化液的抵抗度差，易脱色，故呈黄色或黄棕色 [31]。本实验结果显示，高、中剂量薰衣草全酶提取物可显著减少缺血心肌的染色面积，从而在形态学上说明其对心肌缺血损伤具有明显的保护作用。

心肌缺血时可产生大量的自由基，过多的自由基可激发脂质过氧化反应造成细胞膜的损伤，严重时造成细胞的死亡，MDA 是脂质在氧化性极强的自由基作用下发生氧化反应的终末产物，其含量的高低可反映机体受自由基攻击的严重程度。SOD 是体内清除自由基的重要物质之一，其活力的高低可反映机体清除自由基的能力 [33]。本实验结果显示，高、中剂量薰衣草全酶提取物可显著提高小鼠血清中 SOD 活性，减少 MDA 的产生，提示其可能通过增强心肌清除自由基的能力，对抗心肌损伤的氧化损伤，这也与中药通过提高机体清除氧自由基的能力保护心肌血管抗缺血损伤的观点 [34-35] 相一致。


