

天麻醇提取物对模型小鼠学习记忆的改善作用*

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摘要:目的 探讨天麻醇提取物对模型小鼠学习、记忆的改善作用。方法 将100只小鼠随机分为正常对照组(A组,等体积蒸馏水),模型组(B组,等体积蒸馏水)、吡拉西坦组(C组,700 mg/kg),天麻醇提取物高、低剂量组(D₁组、D₂组,1.0、0.4 g/kg),灌胃给药,灌胃体积为每10 g体质量0.2 mL,每天1次,连续30 d。灌胃结束第2天开始,B组、C组、D₁组、D₂组大鼠均先后予以腹腔注射东莨菪碱(1 mg/kg)、氯霉素(200 mg/kg),以及灌胃20%乙醇(每10 g体质量0.2 mL),依次复制小鼠记忆获得、巩固和再现障碍模型,均每天1次,连续6 d,间隔5 d再进行下个建模。A组小鼠腹腔注射等体积0.9%氯化钠注射液和灌胃给予等体积蒸馏水。行为学检测与建模同时开始,测定小鼠120 s内的逃避潜伏期和空间搜索距离,计算滞留时间、搜索距离百分比。结果 与A组比较,记忆获得障碍模型组、记忆巩固障碍模型组小鼠逃避潜伏期和搜索距离均显著延长,滞留时间百分比和搜索距离百分比均显著降低,记忆再现障碍模型组小鼠逃避潜伏期显著延长($P < 0.01$ 或 $P < 0.05$)。与记忆获得障碍模型组比较,C组、D₁组和D₂组小鼠的逃避潜伏期和搜索距离均显著缩短($P < 0.01$ 或 $P < 0.05$);与记忆巩固障碍模型组比较,C组、D₁组和D₂组小鼠的逃避潜伏期和搜索距离均显著缩短,C组和D₁组小鼠的滞留时间百分比和搜索距离百分比均显著升高($P < 0.01$ 或 $P < 0.05$);与记忆再现障碍模型组比较,C组和D₁组小鼠的逃避潜伏期显著缩短($P < 0.01$)。结论 天麻醇提取物对模型小鼠学习、记忆有一定改善作用。

关键词:天麻醇提取物;东莨菪碱;氯霉素;乙醇;改善记忆;Morris水迷宫

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Improvement Effect of Alcohol Extract of *Gastrodiae Rhizoma* on Learning and Memory of Model Mice

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Abstract: Objective To investigate the improvement effect of alcohol extract of *Gastrodiae Rhizoma* on learning and memory of model mice.

Methods Totally 100 mice were randomly divided into the normal control group (group A, equal volume of distilled water), model group (group B, equal volume of distilled water), piracetam group (group C, 700 mg/kg), alcohol extract of *Gastrodiae Rhizoma* high dosage group and low dosage group (group D₁ and group D₂, 1.0 and 0.4 g/kg), all mice were administrated by gavage, the volume was 0.2 mL/10 g of body weight, once a day for 30 d. From the second day after administration, the mice in group B, group C, group D₁ and group D₂ were given intraperitoneal injection of scopolamine (1 mg/kg), chloramphenicol (200 mg/kg), and given intragastric administration of 20% ethanol (0.2 mL/10 g of body weight), and then the memory acquisition impairment model, memory consolidation impairment model, memory reappearance impairment model were reproduced, once a day for 6 d, and then the next models were established at 5 d intervals. The mice in group A were given intraperitoneal injection of 0.9% Sodium Chloride Injection and intragastric administration of equal volume of distilled water. At the same time, the behavioral detection and modeling were started. Escape latency and spatial search distance were measured within 120 s, the retention time and percentage of search distance were calculated. **Results** Compared with those in group A, the escape latency and search distance of mice were significantly prolonged, while the retention time and percentage of search distance were significantly reduced in the memory acquisition impairment model group and memory consolidation impairment model group, and the escape latency of mice in the memory reappearance impairment model group was significantly prolonged ($P < 0.01$ or $P < 0.05$). Compared with those in the memory consolidation impairment model group, the escape latency and search distance of the mice in group C, group D₁ and group D₂ were significantly shortened ($P < 0.01$ or $P < 0.05$). Compared with the memory consolidation impairment model group, the escape latency and search distance of mice in group C, group D₁ and group D₂ were significantly shortened, while the retention time and percentage of search distance of mice in group C and group D₁ were significantly increased ($P < 0.01$ or $P < 0.05$). Compared with that the memory reappearance impairment model group, the escape latency of mice in group C and group D₁ was significantly shortened ($P < 0.01$). **Conclusion** Ethanol extract of *Gastrodiae Rhizoma* can improve the learning and memory of model mice.

Key words: ethanol extract of *Gastrodiae Rhizoma*; scopolamine; chloramphenicol; ethanol; memory improvement; Morris water maze

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天麻,又名赤箭,作为我国名贵中药,可作为保健食品的材料,并进入了食药物质目录^[1]。《神农本草经》记载:“赤箭辛,温,无毒,久服益力气,长阴肥健,轻身增年。”本课题组前期研究表明,天麻乙酸乙酯提取物可改善模型小鼠学习能力,降低脑内胆碱酯酶的活性^[2],提示天麻可辅助改善记忆^[3]。本实验中对天麻醇提取物在改善记忆方面的效果进行了评价^[3]。现报道如下。

1 材料与方法

1.1 动物、仪器与试剂

动物:清洁级昆明种小鼠 100 只,体质量(20±2)g,雌雄各半。由湖南斯莱克景达实验动物有限公司提供,动物生产许可证号 SCXK(湘)2019-0004。标准条件饲养,自然进食、饮水,适宜温度、湿度,人工黑暗/光照交替。

仪器:TM-Vision 型 Morris 行为学水迷宫(成都泰盟软件有限公司)。

试剂:天麻醇提取物(自制);吡拉西坦片(杭州民生药业有限公司,批号为 20171204,规格为每片 0.4 g);氢溴酸东莨菪碱注射液(遂成药业股份有限公司,批号为 2017110908,规格为每支 1 mL:0.3 mg);氯霉素注射液,国药集团容生制药有限公司,批号为 151108,规格为每支 0.25 g:2 mL);0.9% 氯化钠注射液(广西裕源药业有限公司,批号为 P15070303,规格为每瓶 100 mL:0.9 g);无水乙醇为分析纯,水为蒸馏水。

1.2 方法

分组、给药及建模:将 100 只小鼠随机分为正常对照组(A 组,等体积蒸馏水),模型组(B 组,等体积蒸馏水),吡拉西坦组(C 组,700 mg/kg),天麻醇提取物高、低剂量组(D₁组、D₂组,1.0,0.4 g/kg),灌胃给药,灌胃体积为每 10 g 体质量 0.2 mL,每天 1 次,连续 30 d。灌胃结束第 2 天开始,B 组,C 组、D₁组、D₂组大鼠均先后予以腹腔注射东莨菪碱(1 mg/kg)、氯霉素(200 mg/kg),以及灌胃 20% 乙醇(每 10 g 体质量 0.2 mL),依次复制小鼠记忆获得、巩固和再现障碍模型^[4],均每天 1 次,连续 6 d,间隔 5 d 再进行下个建模。A 组小鼠腹腔注射等体积 0.9% 氯化钠注射液和灌胃给予等体积蒸馏水。

Morris 水迷宫试验^[5-7]:行为学检测与建模同时开始,共进行 6 d,第 1~2 天为训练期,第 3~5 天为测试期,以小鼠平均游泳时间作为衡量其学习记忆能力的指标,第 6 天为空间探索测试期,判断小鼠学习记忆和认知能力。测定小鼠 120 s 内的逃避潜伏期和空间搜索距离,计算滞留时间、搜索距离百分比。

1.3 统计学处理

采用 SPSS 21.0 统计学软件分析。计量资料以 $\bar{X} \pm s$ 表示,行 *t* 检验。*P* < 0.05 为差异有统计学意义。

2 结果

2.1 对记忆获得障碍模型小鼠学习记忆行为的影响

与 A 组比较,B 组小鼠逃避潜伏期、搜索距离均显著延长,滞留时间百分比、搜索距离百分比均显著降低(*P* < 0.01 或 *P* < 0.05);与 B 组比较,C 组、D₁组、D₂组逃避潜伏期和搜索距离均显著缩短(*P* < 0.01 或 *P* < 0.05)。详见表 1。

表 1 天麻醇提取物对记忆获得障碍模型小鼠学习记忆行为的影响($\bar{X} \pm s, n = 10$)

组别	剂量(g/kg)	逃避潜伏期(s)	搜索距离(mm)	滞留时间百分比(%)	搜索距离百分比(%)
A 组		45.52 ± 37.15	7 683.34 ± 6 777.04	48.24 ± 18.40	45.47 ± 14.12
B 组		69.17 ± 32.18 [#]	14 112.71 ± 12 558.78 [#]	30.06 ± 12.16 [*]	31.51 ± 11.38 [*]
C 组	0.7	44.58 ± 31.28 ^{**}	9 494.02 ± 6 914.40 [*]	45.29 ± 12.91 [*]	44.34 ± 12.96 [*]
D ₁ 组	1.0	40.01 ± 30.31 ^{**}	8 654.31 ± 6 772.38 ^{**}	38.74 ± 10.85	37.98 ± 11.97
D ₂ 组	0.4	49.64 ± 36.71 ^{**}	10 649.15 ± 8 150.05 [*]	27.42 ± 9.11	27.27 ± 8.96

注:与 A 组比较,[#]*P* < 0.05,^{**}*P* < 0.01;与 B 组比较,^{*}*P* < 0.05,^{**}*P* < 0.01。表 2、表 3 同。

2.2 对记忆巩固障碍模型小鼠学习记忆行为的影响

与 A 组比较,B 组小鼠逃避潜伏期、搜索距离均显著延长,滞留时间百分比、搜索距离百分比均显著降低(*P* < 0.01 或 *P* < 0.05);与 B 组比较,C 组、D₁组、D₂组小鼠逃避潜伏期和搜索距离均显著缩短,C 组和 D₁组小鼠滞留时间百分比和搜索距离百分比均显著升高,*P* < 0.01 或 *P* < 0.05。详见表 2。

表 2 天麻醇提取物对记忆巩固障碍模型小鼠学习记忆行为的影响($\bar{X} \pm s, n = 10$)

组别	剂量(g/kg)	逃避潜伏期(s)	搜索距离(mm)	滞留时间百分比(%)	搜索距离百分比(%)
A 组		47.40 ± 37.76	8 873.59 ± 7 494.09	43.67 ± 5.24	42.81 ± 5.92
B 组		86.12 ± 30.01 [#]	18 508.23 ± 11 687.15 [#]	24.75 ± 3.80 [#]	24.82 ± 5.43 [#]
C 组	0.7	67.12 ± 43.14 ^{**}	9 675.37 ± 5 486.20 ^{**}	33.73 ± 7.84 ^{**}	33.58 ± 11.26 [*]
D ₁ 组	1.0	60.67 ± 39.62 ^{**}	9 335.49 ± 6 721.76 ^{**}	42.56 ± 18.92 ^{**}	41.18 ± 16.61 ^{**}
D ₂ 组	0.4	70.03 ± 41.42 [*]	11 523.89 ± 8 714.35 ^{**}	30.60 ± 8.64	28.88 ± 5.79

2.3 对记忆再现障碍模型小鼠学习记忆行为的影响

与 A 组比较,B 组小鼠逃避潜伏期显著延长(*P* < 0.01);与 B 组比较,C 组和 D₁组小鼠逃避潜伏期显著缩短(*P* < 0.01)。详见表 3。

表 3 天麻醇提取物对记忆再现障碍模型小鼠学习记忆行为的影响($\bar{X} \pm s, n = 10$)

组别	剂量(g/kg)	逃避潜伏期(s)	搜索距离(mm)
A 组		19.62 ± 15.57	4 952.60 ± 6 162.81
B 组		95.64 ± 41.80 [#]	5 157.65 ± 3 823.93
C 组	0.7	70.35 ± 47.52 ^{**}	6 994.12 ± 9 310.66
D ₁ 组	1.0	65.39 ± 47.48 ^{**}	5 925.89 ± 5 267.37
D ₂ 组	0.4	87.03 ± 43.68	7 176.87 ± 5 853.73