The Mechanism of Plant Drug Ameliorating Depression-like Behavior in PCPA Model Mice

[Abstract] Objective: To preliminarily investigate the effect and mechanism of Plant Drug on depression-like behavior in PCPA model mice. Methods: The experiment was divided into control group, model group and Plant Drug group. The effect of Plant Drug on depression-like behavior in PCPA model mice was tested by behavioral methods of open field, tail suspension and forced swimming. Results: The open field experiment showed that the central activity distance and time of the model group were decreased compared with the control group. Compared with the model group, the two indexes of central activity distance and time were improved in the Plant Drug group. The results of tail suspension experiment showed that compared with the control group, the immobility time of the model group was significantly increased. Compared with the model group, the immobility time of mice in the treatment group was significantly reduced. The results of forced swimming experiment showed that compared with the control group, the immobility time of the model group was significantly increased. Compared with the model group, the immobility time of mice in the drug group was significantly reduced. Conclusion: Plant Drug can improve depression-like behavior in PCPA mice.

[Key words] Plant Drug; depression; ethology

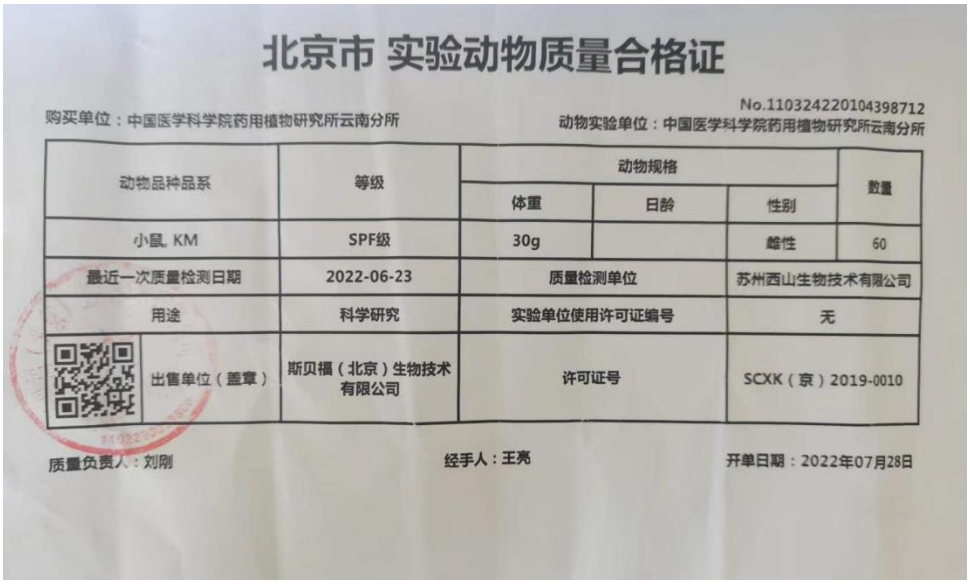
Depression is a common mental illness and a major cause of sub-health and disability worldwide, affecting nearly 300 million people [1]. It is characterized by persistent symptoms, including low mood, decreased pleasure, suicidal thoughts, psychomotor retardation or agitation, changes in appetite and sleep patterns, decreased energy or increased fatigue, inattention, difficulty in decision making, irritability or physical restlessness, and slower speech or movement [2]. The existence and severity of symptoms vary from individual to individual. Patients with depression have a higher rate of other physical diseases, decreased social function and increased mortality [3]. The complexity of the disorder is compounded by the fact that it often co-occurs with other mental illnesses. Therefore, it is of great medical and social significance to study the etiology, pathogenesis and treatment of depression and improve the living standard of patients. At present, the pathogenesis of depression is mainly based on the monoamine neurotransmitter hypothesis. The monoamine neurotransmitters and their metabolites in the brain mainly include norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT), and their content changes are closely related to the pathogenesis of depression. 5-HT plays a key role in antidepressant regulation and can regulate various physiological responses in the central nervous system and peripheral nervous system [4]. Dl-4-chlorophenylalanine (PCPA) is a 5-HT synthesis inhibitor, which blocks the synthesis of 5-HT by inhibiting tryptophan hydroxylase. When 5-HT is depleted in the brain, depressive symptoms will occur [5].

In this study, PCPA depression model was used to study the antidepressant effect of Plant Drug. Open field, tail suspension and forced swimming experiments were used to verify the effect of Plant Drug on depression-like behavior in PCPA model mice. This study provides experimental basis for the clinical treatment of depression with Plant Drug.

1. Materials and Methods

1.1 Experimental animals

Thirty Kunming strain female mice, aged 16 weeks, weighing 30±5g, were provided by Sibeifu (Beijing) Biotechnology Co., LTD., License number: SCXK (Beijing) 2019-0010. Feeding temperature 22-26℃, humidity 40%-70%. The experimental procedures are in accordance with the regulations.



北京市 实验动物质量合格证

No.110324220104398712

购买单位：中国医学科学院药用植物研究所云南分所　　动物实验单位：中国医学科学院药用植物研究所云南分所

动物品种品系	等级	动物规格			数量
		体重	日龄	性别	
小鼠, KM	SPF级	30g		雌性	60
最近一次质量检测日期	2022-06-23	质量检测单位		苏州西山生物技术有限公司	
用途	科学研究	实验单位使用许可证编号		无	
出售单位（盖章）	斯贝福（北京）生物技术有限公司	许可证号		SCXK（京）2019-0010	

质量负责人：刘刚　　经手人：王亮　　开单日期：2022年07月28日

1.2 Drug

Plant Drug (homemade), in line with the standard of medicinal materials.

1.3 Main reagents and instruments

PCPA (purity > 98%, lot No. D831376) was purchased from Shanghai MacLean Biochemical Technology Co., LTD. Tail hanging and forced swimming are self-made equipment; The open field is provided by Anhui Zhenghua Biological Equipment Co., LTD.

1.4 Animal model copy, grouping and methods

Thirty mice were randomly divided into control group, model group and Plant Drug group, with 10 mice in each group. After 7 days of adaptive feeding, except for

control group, mice in each group were intraperitoneally injected with Dl-4-chlorophenylalanine (PCPA, 100 mg/kg), once a day for 4 consecutive days. After modeling, the administration group was given gavage (6.5g/kg) for 7 days.

1.5 General Status Observation

Before the behavioral test, we observed the general state of each group of mice, including activity, reaction sensitivity, hair gloss and other aspects.

1.6 Ethology

1.6.1 Open field experiment

The outer dimension of the open field test chamber is 105cm×105cm, and the bottom wall and the surrounding side wall are made of black plastic. The experiment was carried out in a quiet environment. The mice were placed in the center of the bottom of the mouse open field box, and cameras and timing were performed at the same time. Observe for 5 minutes and stop the camera. At the end of the experiment, the inner wall and bottom surface of the open field box were cleaned with alcohol to prevent it from affecting the test results of the next animal. At the end of the experiment, the central activity time (s) and central activity distance (cm) of each mouse were analyzed.

1.6.2 Forced swimming experiment

Each group of mice was placed in a cylindrical container with a diameter of 10cm and a water surface height of about 12cm, and the water temperature was 20-25℃. When each mouse, the water surface height was based on the mouse's hind paws not touching the ground and the head just above the water surface. The forced swimming experiment was performed for 6 minutes, and the immobility time of the mice within 4 minutes after the recording was recorded.

1.6.3 Tail suspension experiment

The self-made TST equipment was placed on a 45cm high iron frame and fixed on the bracket in the box with elastic rope at the end of the tail about 1 cm away from the end, keeping the head of the mouse about 20cm away from the ground. The excrement of each mouse was cleaned up after the test, and the environment was kept quiet throughout the test. The mice were kept in a suspended state for 6 minutes and the time of immobility within 4 minutes after the analysis was recorded.

1.7 Statistical analysis

After data collection, SPSS 21.0 software was used for analysis, and data were expressed as ($\bar{x} \pm$ S). Data consistent with normal distribution and overall homogeneity of variance were analyzed by oneway-ANOVA, and $P<0.05$ indicated significant differences.

2 Results

2.1 General status observation

control

model

Plant Drug

FIG. 1 General state of mice in each group

Compared with the control group, the activity of mice in the model group was reduced, the response sensitivity was poor, and the hair gloss was poor. Compared with the model group, the activity of mice in the Plant Drug group was increased, the response sensitivity was better, and the hair gloss was better.

2.2 Open field test results

The open field experiment results showed that, as shown in Table 1, compared with the control group, the central activity distance and central activity time of the model group were reduced. Compared with the model group, the central activity distance and central activity time of the Plant Drug group were increased.

Table 1 Effect of Plant Drug on central activity distance (cm) and central activity time (s) of OFT in PCPA mice

Group	Central activity distance （cm）	Central activity time （s）
control	253.21±43.03	11.60±3.01
model	139.54±37.85	6.77±1.98
Plant Drug	206.06±43.14	9.81±4.26

2.3 Tail suspension test results

The results are shown in Table 2. Compared with the control group, the immobility time of the model group was increased. Compared with the model group, the immobility time of mice in the drug group was reduced.

Table 2 Effects of Plant Drug on TST immobility time in PCPA mice

Group	the immobility time (s)
control	44.39±7.70
model	117.36±19.05
Plant Drug	92.80±19.46

2.4 Results of forced swimming experiment

The results showed that, as shown in Table 3, compared with the control group, the immobility time of the model group was increased; Compared with the model group, the immobility time of mice in the Plant Drug group was reduced.

Table 3 Effects of Plant Drug on FST immobility time in PCPA mice

Group	the immobility time (s)
control	33.07±11.61
model	50.87±17.54
Plant Drug	43.78±18.29

3 Conclusion

Depression is a common clinical mood disorder, with fatigue, low emotion, slow thinking, will activity decline as the main symptoms. However, 5-hydroxytryptamine (5-HT) and its receptor play an important role in the development of depression. Most patients with depression have abnormal metabolism of 5-HT and related products, resulting in the destruction of the balance between some neurotransmitters and endocrine networks [6].

PCPA is a selective and irreversible inhibitor of the initiation enzyme and the rate-limiting enzyme -tryptophan hydroxylase in 5-HT biosynthesis, which can block the synthesis of 5-HT by inhibiting tryptophan hydroxylation and significantly reduce the content of 5-HT in the brain and peripheral blood [7]. 5-HT plays a key role in the regulation of depression, and the reduction of 5-HT content will cause depression [8]. In this study, the depression model was established by intraperitoneal injection of PCPA, and the open field test, tail suspension test and forced swimming test were used to evaluate the depression of mice. The results showed that the number of autonomous activities of mice in PCPA model group significantly decreased, the immobility time of tail suspension and forced swimming significantly increased, and the depression was aggravated. After the administration of Plant Drug, the autonomous activity of mice significantly increased, the tail hanging and forced swimming immobile time significantly decreased, and the depression was significantly alleviated, suggesting that the Plant Drug has a certain alleviating effect on the autonomous activity ability and depression of depressed mice, which provides a theoretical basis for the clinical treatment of depression.

References:

[1] Su Kuan-Pin，Lai Hsueh-Chou，Peng Cheng-Yuan et al. Interferon-alpha-induced depression: Comparisons between early- and late-onset subgroups and with patients with major depressive disorder.[J] .Brain Behav Immun， 2019， 80: 512-518.

[2] World Health Organization. Depression and other common mental disorders global health estimates，Geneva，World Health Organization，2017.

[3] Hwang Hye Jean ， Towards Modern Depressive Disorder: Professional Understanding of Depression in Interwar Britain.[J] .Uisahak， 2019， 28: 787-820.

[4] Bogren M， Brådvik L， Holmstrand C， et al. Gender differences in subtypes of depression by first incidence and age of onset: a follow-up of the Lundby population[J]. European archives of psychiatry and clinical neuroscience，2018，268(2):179-189.

[5] Anne Venner,Roberto De Luca,Lauren T Sohn,et al.An Inhibitory Lateral Hypothalamic-Preoptic Circuit Mediates Rapid Arousals from Sleep[J].Current biology,2019,29(24) :4155-4168.

[6] JOUVET M.Sleep and serotonin:an unfinished story[J].Neuropsychopharmacology,1999,21(2):24S-27S.

[7] Guo H B, WANG H. Application of p-chlorophenylalanine in animal model of insomnia [J]. Chinese Journal of Comparative Medicine,2019,29(6):135-140.GUO Haibo,WANG Hui.Application of Para-chlorophenylalanine in animal models of insomnia[J].Chinese Journal of Comparative Medicine,2019,29(6):135-140.

[8] MURRAY N M,BUCHANAN G F,RICHERSON G B.Insomnia caused by serotonin depletion is due to hypothermia[J].Sleep,2015,38(12):1985-1993.