

## The Protective Effect of extracts from *Dioscorea Macrophylla* on PC12 cell Injury Induced by A $\beta$ 25-35

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**Abstract:** Objective To study the protective effect and mechanism of PC12 cell injury induced by A $\beta$ 25 - 35. Methods: in order to screen out the effective protective concentration of A $\beta$ 25 - 35 induced PC12 cell injury, MTT method was used to detect the apoptosis rate of PC12 cell induced by A $\beta$ 25 - 35 by flow cytometry. In order to test the effect of *Dioscorea macrophylla* on the activity of cholinergic receptor induced by A $\beta$ 25 - 35 in PC12 cells, the matching elements were used to test the effect. Results: MTT showed that  $2 \times 10^{-3} \mu\text{mol} / \text{L}$  of chrysanthemum and estradiol and  $2 \times 10^{-3} \mu\text{mol} / \text{L}$  of estradiol significantly blocked the apoptosis of PC12 cells induced by A $\beta$ 25 - 35, and the activities of acetylcholine and acetylcholine transferase increased. Moreover, the activity of acetylcholine sterase ( $P < 0.05$ ) was decreased, and the difference was statistically insignificant ( $P > 0.05$ ). Conclusion: Jack leaf can increase the content of ACH and hybrid, reduce the content of ACH, regulate the activity of related enzymes in the action of line, and play a defensive role in PC12 cells induced by A $\beta$ 25 - 35.

### 1. Introduction

It is also known as big pigtail, qianjinli, qianjinhong, hongyaotou and Baima excrement. As we all know, the ethnic minorities in the southwest are based on the general use of common drugs, general use of drugs, high nutritional and botanical values, its sweet and mild temperature, and flatness, anti-inflammatory, analgesic, hemostatic, and blood stasis effects. For a long time, the demand for a large number of jacks has led to the continuous reduction of wild resources[1]. Dali Town Ba is one of the main varieties of Zhenba town drugs on the market at present. It is the main raw material of self-made traditional Chinese medicine such as Qianjin pill, Jinji capsule and Zhuangyao Jianshen pill produced by gynecology department. This paper reviews the genetic resources, breeding, cultivation techniques, chemical composition, pharmacological effects and genetic diversity of Jack's leaves, as well as the prospect of further research on Jack's leaves through biotechnology. More than 40 legumes have been found. This genus is widely used in Southeast Asia. Among them, there are 17 variants in southwest, middle and Southeast China. In addition, the organ structure of "Chinese Zhuang medicine" and "Chinese Yao medicine" is different from that of the same genus: the first is the multi branch root system of big leaf catkin[2]. In addition to the morphological and microscopic features, three kinds of invasive Jack hammers, such as jack fruit, can be identified for the purpose of analyzing microscope use.

### 2. Materials and Methods

#### 2.1. Materials

Big leaf Jack (purity 98%, batch number must ), purchased by Beijing Technology estradiol was purchased from simmmaadrezi, dmym high glucose medium, cow serum (FBS) and phosphoric acid buffer solution from Beijing Biotechnology A $\beta$ 25-35; Acetylcholine (ACH) assets,

acetylcholinesterase kit acetylcholinesterase (ChAT) were purchased by Nanjing Jiancheng biotechnology society, and attACHED to FT /PI apoptosis discovery workpiece purchased from Beijing Biotechnology Society.

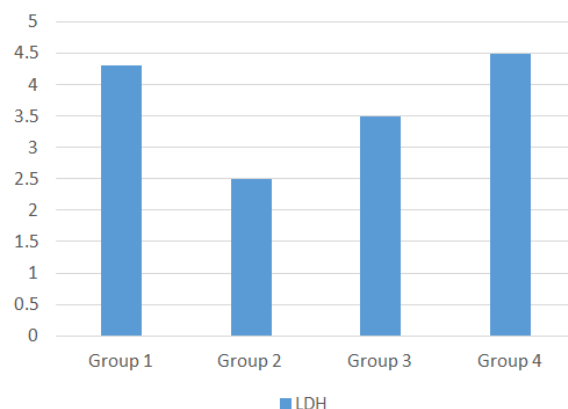


Figure 1 LDH level in cell culture medium of eACH group

## 2.2. Main Instruments

HF 90 carbon dioxide incubator Shanghai made Analytical Instrument Co., Ltd. of China, clean bench of China biology Suzhou Technology.Olympus reverse microscope, Olympus malpractice Club of Japan, micro plate Shanghai Thermal Power Instrument of China. Automatic electrothermal steam sterilization, Shenyang medical instrument factory, Shanghai, China. The UV UF ultra pure water preparation system, Haitai instrument, Shanghai, China. Model 8 - 5K centrifugation, Newton scientific instrument factory, Shanghai, China. Hpc-150 personal portable flow meter is purchased from the company.

## 2.3. Method

Log phase 4000 dictation / HDF was inoculated and cultured at 37°C and 5%. The experiment was divided into blank group (normal PC12 cells), model group, positive control group (estradiol group) and administration group. Xiaoyin group (4 ml DMEM culture medium for 24 hours); DMEM culture medium 3 ml, 20 μmol/L Aβ25 - 35, 24 hours culture); positive control group of estradiol in DMEM  $2 \times 10^{-5}$  μmol/L,  $2 \times 10^{-4}$  μmol/L,  $2 \times 10^{-3}$  μmol/L DMEM culture medium  $2 \times 10^{-7}$  μmol / L,  $2 \times 10^{-6}$  μmol/L DMEM culture medium for 2 hours, 20 μmol/L Aβ25 - 35, 24 hours culture. EACH group has six composite holes. MTT test: 96 μm of Weier plate with 5 g/L concentration, plus 4 hours, the turbid liquid was pushed out, and 150 DMSO was discarded for ACH dictation dissolution, plus 37°C for 10 hours at a certain temperature. The absorbance at 490nm was measured on a microplate reader. The apoptosis rate was detected by flow cytometry. PC12 cells were seeded on the 6well plate[3]. After 24 hours of culture, leaves of  $2 \times 10^{-3}$  μmol/L and estradiol of  $1.5 \times 10^{-9}$  mol / L were added to the drug administration group for positive control. Four hours later, 20 μmol / l Aβ was added to the administration group, estradiol group and Aβ group, and the same amount of culture medium was added to the blanking group, and the cells were collected 22 hours later. The cells were resuspended by adding 195 μl of aflatoxin V-FITC binding solution, and 5 μL and 10 μl of propionic iodine (PI) staining solution of aflatoxin were added. The apoptosis rate of cells was detected by flow cytometry after 15 minutes of incubation in the vagina. The ACH and ACH chat survival rate of PC12 cells were measured 24 hours after injection. The cells were collected thoroughly by cell antiskid rod, sonicated, centrifuged at 4°C, 2500 R / min for 10 minutes, and separated.

## 3. Statistical Analysis

For statistical analysis, SPSS 21.0 software is used [4]. The experimental data are expressed by

$\bar{X} \pm s$ . The difference was  $p < 0.05$ . Statistically important.

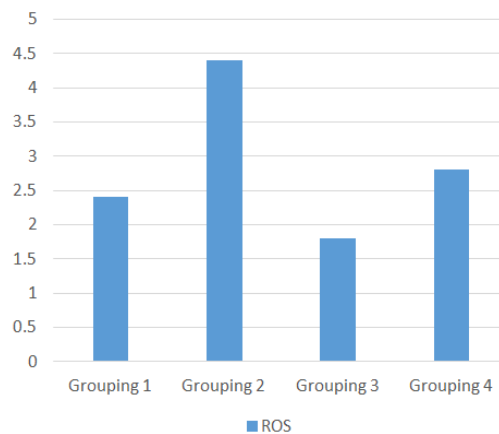


Figure 2 ROS level in cell culture medium of eACH group

#### 4. Results

PC12 cells induced by A $\beta$ 25 - 35 were treated with a large leaf Jack[5]. According to the effective concentration of 25.35 to induce PC12 cell injury, the cell proliferation rate also increased significantly. In this experiment, was used to show the effect of Dioscorea macrophylla on the apoptosis rate of PC12 cells induced by A $\beta$ 25 - 35. Compared with the blanking group, the apoptosis rate of PC12 cells (model group) induced by A $\beta$ 25 - 35 increased significantly ( $P < 0.01$ ). The apoptosis rate of PC12 cells ( $P < 0.01$ ), but the difference between the two groups was not statistically significant. The inhibition of PC12 cells induced by A $\beta$ 25 - 35 was shown in the large leaf extract. In PC12 cells induced by A $\beta$ 25 - 35, compared with ACH, ACHE and the group of clearing, ACH and chatting, the activity of ACHE decreased significantly, and the activity of ACHE increased significantly ( $P < 0.01$ ). Compared with the model group, the ACH and activity of E2 + A $\beta$ 25-35 group and E2 + A $\beta$ 25-35 group increased significantly, and the ACHE activity decreased significantly ( $P < 0.01$ ), but the difference between the two groups was not statistically significant. Effective ( $P > 0.05$ )[5]. This indicated that the jack increased the activity of ACH and decreased the ACHE activity in PC12 cells which were injured to E12 by the same A $\beta$ 25 - 35.

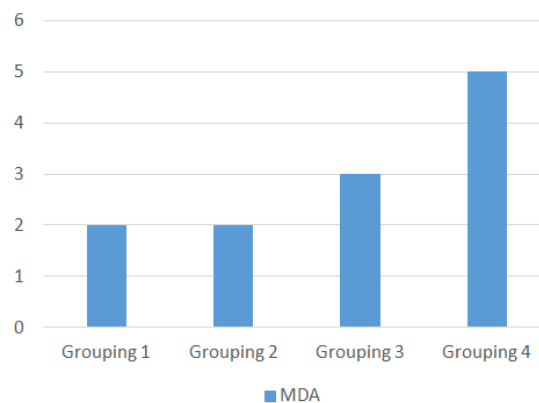


Figure 3 MDA level in cell culture medium of eACH group

#### 5. Debate

According to the epidemiological survey, there are now 1.5 million AD patients in the world[6]. The World Health Organization predicts that the average incidence of ad is about 3% in people over 60 years old and about 30% in people over 85 years old. It is estimated that there will be 141.4 billion AD patients in the world by 2050. The main clinical symptoms of AD are progressive

memory disorder, cognitive dysfunction, personality changes, language disorders and other neuropsychological symptoms, which will have a great impact on social, professional and life functions, and eventually die within 3 to 10 years. Stroke is the fourth leading cause of death. China is rapidly entering an aging society, which is the most important public health issue in China every year. The cause and cause of ad is unknown[7]. At present, there are waterfall hypothesis, cholinergic injury hypothesis, calcium overload hypothesis. In recent years, it is believed that the cause of ad is cholinergic disorder. The functional defect of cholinergic system is one of the causes of ad which is known by academe. It is also the physiological basis of long-term memory. Improving ace synthesis or reducing ACH decomposition can affect learning and memory function. Studies have shown that when quinone chat activity increases, ACH activity in the frontal cortex decreases. It is speculated that ketones block ACHE activity, increase chat activity, decrease ACH decomposition and increase synthesis in cerebral cortex of ovariectomized rats. This can improve the learning and memory of mice[8]. At present, there are few reports on the biological and pharmacological activities of *Dioscorea macrophylla* in China. In the current study, data show the complications of antioxidants, anti-inflammatory drugs, analgesics, and diabetes. The activities of ACH, chat and ACHE in PC12 cells were investigated. The results showed that the ACHE content and chat activity of PC12 induced by A $\beta$ 25 - 35 decreased significantly, while ACHE activity increased significantly. This tendency is consistent with the theory of choline action. Previous studies have shown that there are many traditional Chinese medicine on ACHE and hydrogenase. Saponins protect the central nervous system by increasing content and chatting activities. Through central cholinergic action, the decoction significantly increased ACH and chat activity, reduced ACHE activity, and protected the central cholinergic injury caused by AD. Increase the activity of ACE, promote the synthesis of ACH, improve the learning and memory function of dementia patients. It is suggested that the anti-aging effect of black soybean juice is related to the decrease of ACHE activity and the improvement of central cholinergic system. Propanol extract and flavonoids are codeine. Acetylcholine concentration and acetylcholine receptor activity are the reports of ACH activity induced by scopolamine in the cerebral cortex and hippocampus of rats, which can enhance the development of neuromuscular junction. Presynaptic membrane receptor is acetylcholine separation, axonal competition and synaptic regulation clearance. The results of this study showed that Jack larva increased the mixed activity of PC12 cells induced by A $\beta$ 25-35, hindered ACHE activity, promoted ACH synthesis, hindered ACH decomposition, and increased ACH content in tissues [9]. The decrease of cholinergic injury induced by A $\beta$ 25-35 in PC12 cells

## 6. Conclusion

All of them protected PC12 cells induced by A $\beta$ 25 - 35 by increasing ACH and chat content, reducing ACHE content, regulating the activity of enzymes related to choline action[10]. This provides the experimental basis for the treatment of ad with the method of "big leaf pulling".

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