

Original Research Article

Effect of *Alisma plantago-aquatica* Linn extract on hyperprolactinemia in rats

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Abstract

Purpose: To investigate the anti-hyperprolactinemia effect and mechanism of action of *Alisma plantago-aquatica* Linn. extract (APLE) in rats.

Methods: The hyperprolactinemia (hyperPRL) model of rats was established by intraperitoneal (i.p.) metoclopramide (200 mg/kg daily) for 10 days. Sixty rats were divided into six groups ($n = 10$ each): normal group, hyperPRL control group, hyperPRL plus 0.6 mg/kg bromocriptine (as a positive control) group, and hyperPRL plus high (14.4 g/kg), medium (7.2 g/kg), or low (3.6 g/kg) dose of APLE. Bromocriptine or vehicle control was administered to the rats daily for 30 days, and the hypothalamus dopamine D2 receptor, protein kinase A (PKA), and cyclic adenosine monophosphate (cAMP) levels were investigated by Western blot.

Results: Compared with the normal rats, hypothalamus dopamine D2 receptor protein expression was significantly lower in hyperPRL rats ($p < 0.01$), but was changed significantly after 30-day doses (various) of APLE administration (3.6 g/kg, $p < 0.05$; 7.2 and 14.4 g/kg, $p < 0.01$). Compared with the control rats, hypothalamus PKA and cAMP levels were significantly higher in hyperPRL rats ($p < 0.01$). These increases in PKA and cAMP were significantly attenuated by 30-day of bromocriptine treatment or various doses of APLE ($p < 0.01$).

Conclusion: The anti-hyperPRL activity of APLE is confirmed from the findings of this study. Thus, the plant can potentially be developed into a new anti-hyperprolactinemia drug.

Keywords: Hyperprolactinemia, Dopamine D2 receptor, cAMP/PKA, *Alisma plantago-aquatica*

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INTRODUCTION

Hyperprolactinemia (hyperPRL) is an hypothalamic-pituitary axis disease which shows that serum prolactin > 25 ng/mL in young women. The symptom of hyperPRL includes galactorrhea, menstrual irregularities, and even infertility [1]. The mobility of hyperPRL is 0.4% in

the normal adult population. However, in menstrual problems women, it becomes 9-17 % [2-3]. In clinical, bromocriptine and cabergoline were the only first-line drugs for the treatment of hyperPRL as dopamine D2 receptor agonists. Although these drugs have achieved significant effect, they bring many adverse effects such as headache, nausea, vomiting and so on. About 12

% of patients are unable to complete a course of bromocriptine [4].

Traditional herbal medicine is usually used for curing many diseases and the curative effects have been confirmed in the world such as China, India and African countries. *Alisma plantago-aquatica* is a medicinal herb used for treating hyperPRL without toxic side effects. In a previous study, the serum prolactin level of rats decreased significantly when administrated of *Alisma plantago-aquatica* extract [5]. However, the anti-hyperPRL mechanism of APLE is yet unknown. In this study, we supposed that the anti-hyperPRL effect of APLE could be mediated by regulating dopamine D2 receptor. Therefore, we designed an animal experiment to study the effect and mechanism of APLE on hyperPRL.

EXPERIMENTAL

Materials

The leaf samples of *Alisma plantago-aquatica* Linn. were bought in Dali City of Yunan Province of China and identified by Professor Lei Yang of Anhui Medical University (Hefei, Anhui province, China). The remaining specimens were saved in the Department of pharmacy, The First People's Hospital of Wuhu City (Number APLE 201710025).

The leaves of *Alisma plantago-aquatica* Linn. samples was washed first and then dried in an oven. The dried *Alisma plantago-aquatica* Linn. was added into a frying pan for decocting in water at 60 °C. It was repeated for three times and for one hour each time. At last, APLE was obtained by concentrating the decoction for several hours [6]. The last APLE concentration was 22.8 mg/mL.

Pharmacodynamics experiment in hyperPRL rats

Female rats (Wistar, weighing 180-200 g) were bought the laboratory animal centre of Anhui medical university, Hefei, Anhui, China. After the rats were kept in the laboratory, they were free access to food and water, and lasted for 7 days before use. All experiments were checked and confirmed by the Animal Care and Use Committee of the First People's Hospital of Wuhu City (approval ref no. 20130514) and carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [7].

The experimental hyperPRL model was established by injecting metoclopramide solution

(MCP, 200 mg/kg daily) into the abdominal cavity of rats for 7 days [8]. Sixty rats were randomly separated into six groups: normal control (non-hyperPRL rats), hyperPRL control rats, positive drug bromocriptine (hyperPRL rats treated with 0.6 mg/kg bromocriptine), and low-dose, medium-dose, and high-dose APLE (hyperPRL rats treated with 3.6, 7.2, and 14.4 g/kg APLE, respectively). All used drugs were dissolved in 2 mL water. The daily bromocriptine and APLE dosages were calculated according to the conversion equation of human and animal. All group rats were administered of 2 mL of distilled water by gavage. The intragastric administration lasted daily for 30 days.

Western blot analysis

After the hypothalamus tissues were collected from the rat brains, they were homogenized in RIPA buffer with protease inhibitor immediately, and then centrifuged at 5000 × g for 10 min at 4 °C. The supernatant samples were separated and obtained by centrifuging at 10000 × g for 15 min. Then the RIPA buffer and loading buffer were added into the protein extracts. Each sample was dissolved by loading buffer, and then was separated by 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis. The polyvinylidene fluorides (Merk) were used for protein transferring. All the membranes were blocked out with 0.1 % Tween-20 and 5 % fat-free milk for 1 h at room temperature, and then incubated with the appropriate primary antibodies (DRD2, 1: 2000; β-actin as loading control, 1 : 3000, Proteintech, China). The immuno-reactive bands were observed by incubation with ECL chemiluminescence reagent (Proteintech, China). The gray value of the color stripes is quantified by AlphaEaseFC 4.0 analysis software. The relative values of DRD2 protein was calculated by normalizing to β-actin levels.

Hypothalamus PKA and cAMP test

Following the isolation of the rat brains, the brain tissues were homogenized immediately and centrifuged for 20 min at 5000 rpm. The PKA and cAMP levels were measured in the supernatant by ELISA kits (Wuhan Huamei biological Co., Ltd., China).

Statistical analysis

All data were expressed as mean ± SEM. One- or two-way analyses of variance (ANOVA) were used to analysis statistical significance, followed by post-hoc multiple comparisons (Student-

Newman-Keuls method). $P < 0.05$ was regarded as statistically significant.

RESULTS

Hypothalamus DRD2 level of rats by Western blot analysis

Compared with control rats, the hypothalamus DRD2 protein expression in hyperPRL rats reduced remarkably ($p < 0.01$). After one month treatment of APLE (3.6 g/kg, 7.2 g/kg, or 14.4 g/kg), DRD2 protein level significantly increased ($p < 0.01$). More, bromocriptine with the dosage of 0.6 mg/kg increased the DRD2 protein of hyperPRL rats ($p < 0.01$; Figure 1).

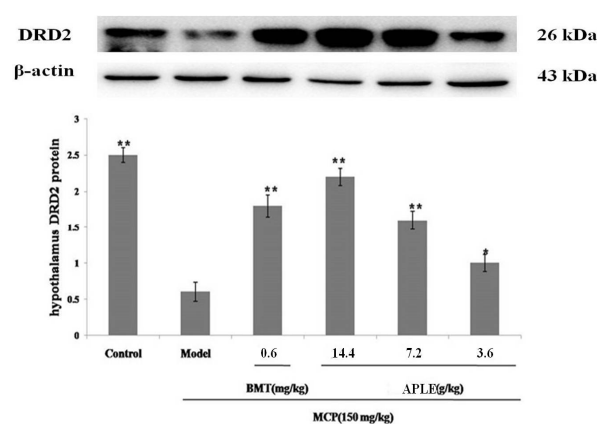


Figure 1: Hypothalamus DRD2 protein analysis by Western blot. After establishing a hyperPRL model by injecting metoclopramide (MCP), rats were treated with bromocriptine (BMT) or APLE at indicated doses for 30 days. $p < 0.05$ and $p < 0.01$ versus hyperPRL group

Hypothalamus PKA and cAMP levels of rats by ELISA

Compared with normal group, hypothalamus PKA levels of control rats significantly increased (0.64 ± 0.03 versus 0.21 ± 0.01 $\mu\text{g/mL}$; $p < 0.01$). These increase of PKA level reversed significantly by treatment with 3.6 g/kg APLE (0.44 ± 0.02 $\mu\text{g/mL}$; $p < 0.05$), 7.2 g/kg APLE (0.38 ± 0.03 $\mu\text{g/mL}$; $p < 0.01$), or 14.4 g/kg APLE (0.24 ± 0.03 $\mu\text{g/mL}$; $p < 0.01$) for 30 days. Likewise, hypothalamus cAMP levels were significantly higher in hyperPRL rats compared with the control group (2.7 ± 0.3 versus 0.34 ± 0.02 ng/mL ; $p < 0.01$). The increases in cAMP levels were significantly attenuated by treatment with 3.6 g/kg APLE (1.7 ± 0.2 ng/mL ; $p < 0.05$), 7.2 g/kg APLE (1.4 ± 0.2 ng/mL ; $p < 0.05$), or 14.4 g/kg APLE (1.3 ± 0.2 ng/mL ; $p < 0.01$) for 30 days.

DISCUSSION

In recent years, many traditional medical plants have been found to treat chronic diseases, such as cancer, hyperuricemia, diabetes and so on. In our previous study, APLE inhibited prolactin secretion of hyperPRL mice significantly. And more, APLE increased hypothalamus DRD2 level and reduced cAMP and PKA expression of hyperPRL rats.

Dopamine receptors are expressed at the cell membrane and are responsible for the signal transduction. They belong to the family of seven transmembrane domain G-protein coupled receptors (GPCR) [9-11]. The D1-like receptor subfamily members (including D1 and D5) couple mostly to G_{α_s} and promote the increase of the second messenger cAMP and the activity of PKA. However, the D2-like subfamily members (including D2, D3, and D4) couple to $G_{\alpha_{i/o}}$ and negatively promote the increase of cAMP and lower PKA level [12-14]. The role of DRD2 has been confirmed in the brain and kidney [15,16].

DRD1 and DRD2 both express in human brains abundantly [17]. It was reported that overexpression of DRD2 in MMQ cell line by adenovirus results in a decrease in intracellular cAMP, resulting the prolactin expression decrease [18,19]. In previous study, many scientists found that bromocriptine inhibited PRL secretion by binding to DRD2 of hypothalamic dopaminergic system, resulting in cAMP/PKA signal transduction pathway activation. Our study result showed that APLE increases hypothalamus DRD2 level and reduced cAMP and PKA expression in the hyperPRL rats. These findings suggest that the anti-hyperPRL activity of APLE was mediated by DRD2/cAMP/PKA signaling pathway.

CONCLUSION

APLE showed anti-hyperPRL activity in rats, suggesting it may be possible to develop into a new drug for the management of hyperPRL.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors. Lin Wang designed the experiments and revised the paper. Ming Wen, Deming Tian, Shao-Feng Shi, Xin-Hua Chen, and Yan-Xiang Zhang performed the experiments and wrote the paper.

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