Tropical Journal of Pharmaceutical Research November 2019; 18 (11): 2411-2414
**ISSN:** 1596-5996 (print); 1596-9827 (electronic)
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Available online at http://www.tjpr.org
**http://dx.doi.org/10.4314/tjpr.v18i11.26**

**Original Research Article**

# Effect of *Acorus tatarinowii* extract on hyperprolactinemia in rats

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*Sent for review: 17 July 2019* *Revised accepted: 25 October 2019*

## Abstract

***Purpose:*** *To determine the mechanism underlying the anti-hyperprolactinemia effect of Acorus tatarinowii extract (ATE) in rats.*
***Methods:*** *Rats were divided into six groups (n =10 each group), viz, healthy control, untreated hyperprolactinemic rats, hyperprolactinemic rats treated with bromocriptine (0.6 mg/kg), and hyperprolactinemic rats treated with ATE (3.2, 6.4, or 12.8 g/kg). After 30 days, the hypothalamic protein levels of dopamine D2 receptor, protein kinase A (PKA), and cyclic adenosine monophosphate (cAMP) were determined.*
***Results:*** *Dopamine D2 receptor levels were lower in untreated hyperprolactinemic rats than in healthy control ($p < 0.01$), but this decrease was attenuated by ATE ($p < 0.05$). Elevated PKA levels in untreated hyperprolactinemic rats (0.78 ± 0.03µg/mL, $p < 0.01$) were decreased by ATE (3.2 g/kg, 0.51 ± 0.02 µg/mL, $p < 0.05$; 6.4 g/kg, 0.39 ± 0.03 µg/mL, $p < 0.01$; 12.8 g/kg, 0.24 ± 0.04 µg/mL, $p < 0.01$). Similarly, elevated cAMP levels in hyperprolactinemic rats (3.1 ± 0.3 ng/mL) were lowered by ATE (3.2 g/kg, 2.2 ± 0.4 ng/mL, $p < 0.05$; 6.4 g/kg, 1.8 ± 0.3 ng/mL, $p < 0.01$; 12.8 g/kg, 1.4 ± 0.3 ng/mL, $p < 0.01$).*
***Conclusion:*** *ATE anti-hyperprolactinemia activity is mediated by dopamine D2 receptor signaling via cAMP/PKA pathway.*

***Keywords:*** *Hyperprolactinemia, Acorus tatarinowii, Dopamine D2 receptor, Bromocriptine, Cyclic adenosine monophosphate, Hypothalamic protein*

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## INTRODUCTION

Hyperprolactinemia is a heterogeneous disorder characterized by elevated blood prolactin level. This disorder of the hypothalamic-pituitary axis can have a physiological cause (e.g., pregnancy), pathological cause (e.g., tumor), or unknown cause. In different patients, similarly elevated prolactin levels can result in severe clinical manifestations or no symptoms at all [1]. Hyperprolactinemia is more common in women than in men. Its prevalence in an unselected adult population is 0.4 % but has been reported

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as 5 % in women visiting a family planning clinic, 9 % in women with adult-onset amenorrhea, and 17 % in women with polycystic ovary syndrome [2,3].

The two most commonly prescribed drugs for the treatment of hyperprolactinemia are bromocriptine and cabergoline. These medications are dopamine receptor agonists with similar characteristics and adverse effects (e.g., headache, nausea, and vomiting), but the frequency and severity of adverse effects appear to be lower for cabergoline. Although both drugs are effective in treating hyperprolactinemia, 12 % patients cannot tolerate bromocriptine [4].

Traditional Chinese medicine uses *Acorus tatarinowii* extract (ATE) to treat hyperprolactinemia without the toxic side effects associated with conventional anti-hyperprolactinemia agents. In a previous study, ATE was reported to decrease prolactin levels in hyperprolactinemic mice [5]. However, the mechanism underlying the anti-hyperprolactinemia effects of ATE required further investigation.

Most conventional anti-hyperprolactinemia agents decrease prolactin secretion through the hypothalamic dopaminergic system, and these effects are mediated via the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway [6]. In this study, we hypothesized that ATE alleviates hyperprolactinemia through modulation of the dopamine D2 receptor. We investigated the effects of ATE on protein levels of dopamine D2 receptor, PKA, and cAMP in the hypothalamus of hyperprolactinemic rats.

## EXPERIMENTAL

### Preparation of ATE

Samples of *Acorus tatarinowii* were collected in May 2017 in Dali City, Yunnan Province, China. Taxonomic identification of the plant was performed by Professor Hui Wang of Shandong University (Jinan, China). A voucher specimen (no. ATE 201708021) was deposited in College of Pharmacy herbarium, Shandong University for future reference.

One batch of *Acorus tatarinowii* was dried in an oven. ATE was prepared by steeping the dried *Acorus tatarinowii* in hot water (60 °C) three times (1 hour each time). The *extract was dried first* in an oven and then freeze-dried. The yield was 76.92 % (1 g powder was obtained from approximately 1.3 g crude sample).

### Animals

Female Wistar rats weighing 200 – 220g were obtained from the Shandong Center for Disease Control and Prevention (Jinan, Shandong). The animals had free access to food and water and were allowed to acclimatize for at least 1 week before experimental procedures. All experiments were approved by the Animal Care and Use Committee of Dezhou people's Hospital (approval ref no. 20130326) and were carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [7].

The rats were treated with metoclopramide (150 mg/kg administered intraperitoneally daily for 10 days), a dopamine inhibitor widely used to generate animal models of hyperprolactinemia [8-9]. A total of 60 rats were divided into six groups (n = 10 per group): rats that did not receive metoclopramide (healthy controls), rats that received metoclopramide only (untreated hyperprolactinemic rats), hyperprolactinemic rats treated with 0.6 mg/kg bromocriptine (positive controls), and hyperprolactinemic rats treated with a high, medium, or low dose of ATE (12.8, 6.4, or 3.2g/kg, respectively). The drugs were dissolved in 2 mL water and administered by intragastric gavage; healthy control and untreated hyperprolactin emic rats received 2 mL distilled water. ATE dosages were calculated from the human dosage based on body surface area. The rats were treated for 30 days.

### Western blot analysis

To determine hypothalamic dopamine D2 receptor protein levels after the 30-day treatment period, rat hypothalamus tissues were homogenized in RIPA buffer with protease inhibitor (Complete EDTA-free protease inhibitor cocktail tablets; Roche Applied Science, Mannheim, Germany) and centrifuged at 3000× g for 15 min at 4 °C. The supernatant was centrifuged at 12,000× g for 20 min at 4 °C, and the samples were then diluted in RIPA buffer and loading buffer. After separation with 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis, the proteins were transferred onto polyvinylidene difluoride membranes (Merck). Nonspecific protein-binding sites were blocked by incubating with phosphate-buffered saline containing 0.1% Tween-20 and 5% fat-free milk for 1 h at room temperature, followed by incubation with primary antibodies against dopamine receptor D2 (1:1000) and ß-actin (1:5000), which served as a loading control. The membranes were then incubated with lumiGLO reagent (Cell Signaling Technology) and

exposed to x-ray film (Kodak, Stamford, CT, USA). Protein bands were quantified by Image J (National Institutes of Health, Bethesda, MD, USA). Relative D2 receptor protein levels were determined by normalization to ß-actin.

### Enzyme-linked immunosorbent assay

To determine hypothalamic cAMP and PKA protein levels after the 30-day treatment, rat brains were stripped of membranes and blood vessels, homogenized, and then centrifuged for 15 min at 3000 rpm. Protein levels of PKA and cAMP in the supernatant were then determined by enzyme-linked immunosorbent assay (ELISA, Nanjing Jiancheng Biological Technology Co., Ltd., China).

### Statistical analysis

Treatment groups were compared by one-way analysis of variance (ANOVA), followed by Student–Newman–Keuls post hoc test for multiple comparisons. Data are expressed as mean ± SEM; $p < 0.05$ was considered significant.

## RESULTS

### Effect of ATE on hypothalamus dopamine D2 receptor levels

Results of western blot analysis showed that hypothalamus dopamine D2 receptor protein levels were considerably lower in untreated hyperprolactinemic rats than in healthy controls ($p<0.01$). As expected, this decrease was attenuated by30-day treatment with 0.6 mg/kg bromocriptine (positive control, $p<0.01$). Treatment with ATE resulted in a dose-dependent increase in dopamine D2 receptor levels (3.2 g/kg, $p < 0.05$; 6.4 g/kg, $p < 0.01$; 12.8 g/kg, $p < 0.01$), as shown in Figure 1.

### Effect of ATE on hypothalamus PKA and cAMP levels

Results of ELISA showed that hypothalamus levels of PKA and cAMP were significantly higher in hyperprolactinemic rats compared with healthy controls ($p < 0.01$). This upregulation of PKA and cAMP protein expression was attenuated by treatment with bromocriptine ($p < 0.01$). Treatment with ATE resulted in a dose-dependent decrease in PKA and cAMP levels (3.2 g/kg, $p < 0.05$; 6.4 g/kg, $p < 0.01$; 12.8 g/kg, $p < 0.01$).

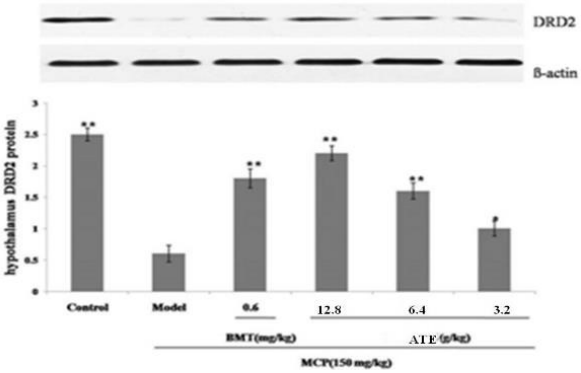


**Figure 1:** Decreased hypothalamus dopamine D2 receptor (DR2) levels in metoclopramide-induced hyperprolactinemic rats (Model) were increased in a dose-dependent manner by ATE. Results are expressed as mean ± SEM (n = 10); $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with untreated hyperprolactinemic rats

## DISCUSSION

In a previous study, ATE significantly decreased prolactin levels in a mouse model of hyperprolactinemia. In the present study, the mechanism underlying the anti-hyperprolactinemia effects of ATE was studied and ATE significantly increased dopamine D2 receptor protein levels and significantly decreased cAMP and PKA protein levels in the hypothalamus of hyperprolactinemic rats.

Dopamine receptors are members of the G-protein coupled receptor family [10-11] and are classified on the basis of their effect on adenylyl cyclase, which regulates the cAMP-PKA pathway. D1-like dopamine receptors (D1 and D5) couple primarily to $G_{\alpha s}$ and increase intracellular levels of the second messenger cAMP and upregulate the activity of PKA. By contrast, D2-like dopamine receptors (D2, D3, and D4) couple to $G_{\alpha i/o}$ and decrease levels of cAMP, thereby suppressing PKA activity [12].

In the adenohypophysis, which secretes prolactin, the predominant dopamine receptor is the D2 receptor [13,14]. In a pituitary cell line transfected with the gene encoding the dopamine D2 receptor, treatment with dopamine decreased intracellular cAMP levels and inhibited prolactin secretion [15,16]. Similarly, most conventional anti-hyperprolactinemia agents are D2 receptor agonists, which decrease prolactin secretion through cAMP/PKA signaling. In this study, ATE increased dopamine D2 receptor levels and decreased cAMP and PKA levels in the hypothalamus of hyperprolactinemic rats significantly. These results suggest that the anti-hyperprolactinemic activity of ATE is mediated by dopamine D2 receptor and cAMP/PKA signaling.

## CONCLUSION

*Acorus tatarinowii* extract decreases prolactin secretion in hyperprolactinemic rats via dopamine D2 receptor and cAMP/PKA signaling. This traditional Chinese medicine may, therefore, be useful for the management of patients with hyperprolactinemia.

## 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. Wan-jing Sun designed all experiments and revised the manuscript. Hong-mei Tang, Jin-zhao Zhao and Xian-jun Meng performed the experiments and wrote the manuscript. Hong Zheng and Fu-tao Zhang contributed equally to this work, and are co-first authors.

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