# Mechanisms of White Peony Root Extract in Polycystic Ovary Syndrome and Its Impact on RNA Methylation: A Meta-Analysis

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Introduction. To systematically review the mechanism of plumbagin in polycystic ovary syndrome (PCOS) and its effect on RNA methylation.

Methods. PubMed, Ovid, Embase, China National Knowledge Infrastructure (CNKI) and Wanfang Data were searched for Chinese and English studies on the mechanism of plumbagin in PCOS and its effect on RNA methylation. The retrieval time was set from the establishment of the database to August January 2024, and RevMan5.3 software was used for Meta-analysis.

Results. A preliminary search obtained 325 articles, and 8 articles were finally included. Eight articles analyzed the mechanism of plumbagin in the treatment of PCOS, and the results showed that plumbagin can improve the ovarian morphology and function of rats, regulate the ovarian hormone microenvironment, and its mechanism may be related to the inhibition of NF-kB signaling pathway activity. Four studies involved the effect of plumbagin on RNA methylation in the treatment of PCOS, and the results showed that plumbagin could reverse RNA methylation and improve the symptoms of PCOS.

Conclusions. Plumbagin can improve the ovarian morphology and function, regulate the ovarian hormone microenvironment, and affect RNA methylation in PCOS rats, which may be related to the inhibition of NF-kB signaling pathway activity. However, given the quantity and quality of the included literature, further research and discussion are needed.

Keywords. Plumbagin; Polycystic ovary syndrome; Mechanism of action; RNA methylation; Ovarian morphology; Ovarian hormone microenvironment; NF-kB signaling pathway

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder with

a high incidence in women of childbearing age, clinically characterized by obesity, amenorrhea, happy androgenism and various ovarian changes [1]. The investigation results of Wang Shujuan et al. [2] showed that the incidence of PCOS was on the rise, accounting for 6%-21% of women of childbearing age, which has become an important cause of anovular-induced infertility in women. According to Qiao Zengfeng et al. [3], the occurrence of PCOS is closely related to chronic low-grade inflammation, and a variety of factors are often involved after the onset of PCOS, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), etc. Therefore, understanding PCOS from the perspective of inflammation and giving TCM intervention can achieve good results [4]. Plumbagin, which contains vanillic acid and plumbagin acid, is present in the whole grass of purple snowflake in the plumbagaceae family. It has strong antibacterial and antiviral effects, and can play a role in eliminating wind and dampness, promoting qi circulation and blood circulation, detoxification and swelling [5]. In recent years, the application of plumbagin has been particularly important, and its use in PCOS has exerted greater social and economic benefits [6]. RNA methylation refers to the chemical modification of RNA methyladenine by the selective addition of methyl groups under the catalysis of methyltransferase, mainly in the form of m6A methylation, but this process is reversible [7]. This study systematically reviews the mechanism of plumbagin in PCOS and its effect on RNA methylation, and reports as follows.

1 Literature screening

1.1 Study DESIGN PubMed, Ovid, Embase, China National Knowledge Infrastructure (CNKI) and Wanfang Database were searched for Chinese and English literatures on the mechanism of plumbagin in PCOS and its effect on RNA methylation. The search time was set from the establishment of the database to August January 2024, and there was no limit to the blinding and allocation scheme.

1.2 Inclusion Criteria (1) All studies were randomized controlled trials in any language. (2) Since the research drugs have not been clinically promoted and applied, the research objects are mainly animals, and the PCOS model has been constructed

[8]. (3) intervention measures. The model control group and blank control group were mainly treated with normal saline by gavage, and the control group was mainly treated with diane-35 / letrozole tablets/metformin. The observation group was treated with plumbagin by gavage. (4) Primary outcome measures: ovarian morphology and function, ovarian hormone microenvironment, RNA methylation and NF-kB signaling pathway.

1.3 Exclusion Criteria (1) repeated publication of literature, review or observational study; (2) Guidelines, expert lecture forums, meeting minutes, news, evidence summary and case reports; (3) It is difficult to extract relevant data and obtain complete data in the literature; (4) Articles published by the same author on different platforms or articles that were difficult to extract one of the main outcome indicators of this study.

1.3 Methods

1.3.1 Search strategy The Chinese and English databases were searched, and the Chinese search terms were plumbagin; Polycystic ovary syndrome; RNA methylation; Ovarian morphology; Ovarian hormone microenvironment; NF-kB signaling pathway; English search terms: Chrysanthin; Polycystic ovary syndrome; RNA methylation; Ovary morphology; Ovarian hormone microenvironment; NF-kB signaling pathway; The search time was set as the establishment of the database to August AND January 2024, and the keywords obtained were searched using Boolean retrieval logic and (AND), logic OR (OR) to arrange and combine the keywords.

1.3.2 Other search methods in order to ensure the completeness and scientificity of the retrieved literature, manual retrieval was performed (as a supplement). When the database literature is difficult to obtain, manual retrieval can be carried out by entering the title, key words, etc., to clarify the literature collection database, and complete the literature retrieval through the included database. For those who are difficult to obtain literature through the above channels, help is sought through the "literature mutual assistance" platform [9].

1.3.3 Chinese search formula the search formula was plumbagin *PCOS* Mechanism of action (ovarian morphology and structure, ovarian hormone microenvironment, NF-kB signaling pathway) *RNA methylation

＃1 Subject headings: PCOS/ all trees/all subsubject headings

＃2 Plumbagin *PCOS* Mechanism of action

＃3 The mechanism of plumbagin in PCOS is analyzed from the perspective of ovarian morphology and structure, ovarian hormone microenvironment, and NF-kB signaling pathway

＃4 Plumbagin *PCOS*RNA methylation

＃5 ＃1OR＃2OR＃3

＃6 Subject headings: influence/all trees/all subheadings

＃7 Effect of plumbagin on the methylation of PCOS

＃8 ＃1OR＃2OR＃3OR＃4OR＃5OR＃6OR＃7OR

1.3.4 English search format The search was for the mechanism of action (ovarian morphology, ovarian hormone microenvironment, NF-kB signaling pathway) *RNA methylation

# 1 Headings: PCOS/ All trees/All sub-headings

# 2 Mechanism of action of Leucanthine *PCOS*

# 3 The mechanism of action in PCOS was analyzed from the perspective of ovarian morphology and structure, ovarian hormone microenvironment, NF-kB signaling pathway, etc

# 4 Chrysanthin *PCOS*RNA methylation

# 5 # 1OR # 2OR # 3

# 6 Headings: Affect/All Trees/All subheadings

# 7 Effect of chrysanthin on methylation of PCOS

# 8 # 1OR # 2OR # 3OR # 4OR # 5OR # 6OR # 7OR

1.4 Study selection and data extraction The main data and content information were extracted, including: the first author, journal, number of cases, publication years, intervention methods, outcome indicators (ovarian morphology and structure, ovarian

hormone microenvironment and NF-kB signaling pathway), etc. Input the obtained data into the form in detail; For the literature that is difficult to download, the authors can be contacted by email or telephone to obtain more data. Literatures that are too old and cannot be downloaded can be excluded [10].

1.5 Literature quality assessment The Newcastle-Ottawa scale (NOS) was used to evaluate the quality of the literature, including research subject selection (4 points), comparability between groups (2 points), and exposure factor measurement (3 points). Cohort studies included study subject selection (4 points), comparability between groups (2 points) and outcome strategy (3 points), with a total score of 18 points, and a score >6 points indicates high quality of the literature [11].

1.6 Statistical methods RevMan5.3 software was used for Meta-analysis. Q test and I2 statistic were used to test the heterogeneity. When P>0.1 and I2<50.0%, the fixed effect model was used, and the random effect model was used to combine the effect values. P<0.05 was considered statistically significant.

## 2 RESULTS

2.1 Literature search A total of 325 literatures were obtained, which were imported into EndNote software, and 121 duplicate literatures were automatically excluded. The titles and abstracts of the remaining literatures were read, and real-time annotation and dynamic deletion were achieved. A total of 36 full-text literatures were downloaded and read, and 8 literatures were finally included, as shown in Figure 1.

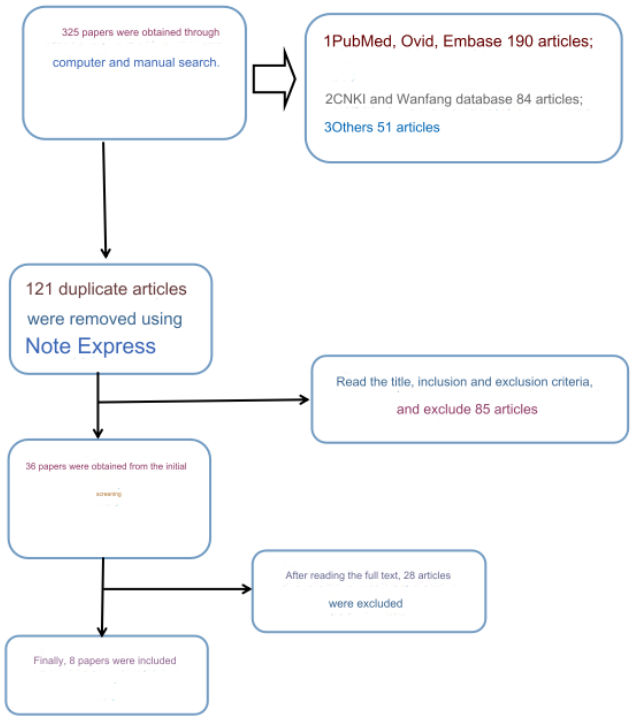


Figure 1 Schematic diagram of the literature search

2.2 Literature data extraction and quality evaluation The included literatures were mainly animals, and the total number of animals was 571. The quality of the literatures was evaluated by NOS scale, and there were 6 high-quality literatures with scores ≥6 points. Two articles scored 5 points and were regarded as low quality, see Tables 1 and 2.

Table 1 Basic characteristics of the literature

| The author | Magazine office | Count only | Number of years | The scheme | Outcome measures |
|---|---|---|---|---|---|
| Yan Xiumin | Journal of Practical Obstetrics and Gynecology | 65 | 2020 | They were randomly divided into control group, model control group, metformin group and plumbagin group, and given corresponding measures of intervention | Serum hormones, inflammatory factors, and RNA methylation |

| | | | | | |
|---|---|---|---|---|---|
| Wu Y | BMC Pregnancy and Childbirth | 72 | 2024 | The rats were randomly divided into blank control group, model control group and plumbagin group, and the corresponding measures were given | Inflammatory factor levels, ovarian hormone microenvironment and NF-kB signaling pathway |
| Huang Z P | International Union of Biochemistry and Molecular Biology Life | 66 | 2010 | Metformin was used as the control group, plumbagin was used as the experimental group, and both groups were given corresponding drug intervention | Ovarian hormone microenvironment, inflammatory factors and RNA methylation |
| Cheng Yao | Chinese Journal of Experimental Medicine | 69 | 2018 | The rats were randomly divided into blank control group, model control group and plumbagin group, and the corresponding measures were given | Ovarian structure and function, RNA methylation |
| Shen L | Annual Review of Biochemistry | 73 | 2014 | The diane-35 control group and plumbagin were used as the experimental group, and both groups were given corresponding drug intervention | Inflammatory factor levels, ovarian hormone microenvironment |
| Chang Zhuang peng | Chinese Journal of Traditional Chinese Medicine | 74 | 2019 | Letrozole tablets were the control group, plumbagin was the experimental group, and both groups were given corresponding drug | Inflammatory factor levels, ovarian hormone microenvironment and RNA methylation |

| | | | | | |
|---|---|---|---|---|---|
| | | | | intervention | |
| Jiang Xiaolin | Journal of Beijing University of Chinese Medicine | 84 | 2018 | The rats were randomly divided into blank control group, model control group and plumbagin group, and the corresponding measures were given | Inflammatory factor levels, ovarian hormone microenvironment |
| Tao Xin | Journal of Sun Yat-sen University (Medical Science Edition) | 68 | 2014 | They were randomly divided into control group, model control group, metformin group and plumbagin group, and given corresponding measures of intervention | Levels of inflammatory factors and NF-kB signaling pathway |

Table 2 Quality evaluation of literature

| The author | Selection OF SUBJECTS | Comparability between groups | Outcome MEASURES | score |
|---|---|---|---|---|
| Yan Xiumin | 2 | 2 | 3 | 7 |
| Wu Y | 2 | 3 | 1 | 6 |
| Huang Z P | 2 | 3 | 1 | 6 |
| Cheng Yao | 2 | 3 | 3 | 8 |
| Shen L | 1 | 2 | 2 | 5 |
| Chang Zhuangpeng | 3 | 3 | 2 | 8 |
| Jiang Xiaolin | 2 | 2 | 1 | 5 |
| Tao Xin | 2 | 2 | 3 | 7 |

2.3 Mechanism of plumbagin in PCOS Eight articles analyzed the mechanism of plumbagin in the treatment of PCOS, with little heterogeneity among different articles. The fixed effect model analysis showed that plumbagin was mainly used in animal experiments in PCOS, which could improve the ovarian morphology and function of

rats and regulate the ovarian hormone microenvironment. The mechanism may be related to the inhibition of NF-kB signaling pathway activity, as shown in Figure 2.

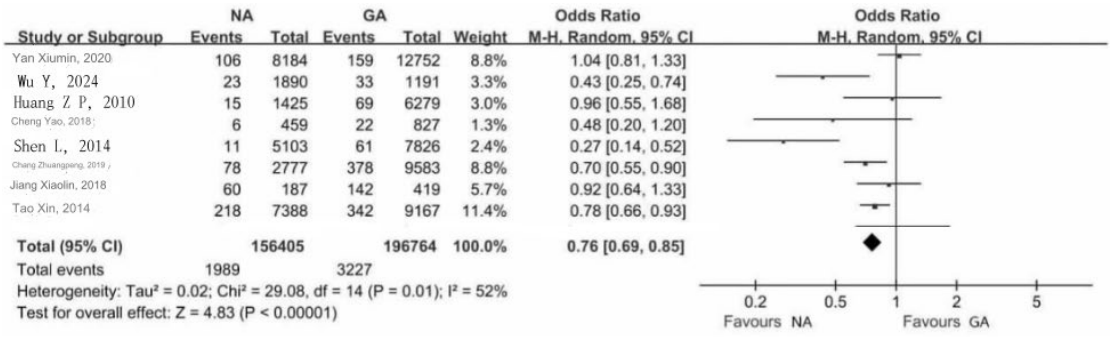


Figure 2 Mechanism of plumbagin in PCOS

2.4 Effect of plumbagin on RNA methylation in PCOS Four studies related to the effect of plumbagin on RNA methylation in the treatment of PCOS, with little heterogeneity among different studies. The fixed effect model analysis showed that plumbagin could reverse RNA methylation and improve the symptoms of PCOS, as shown in Figure 3.

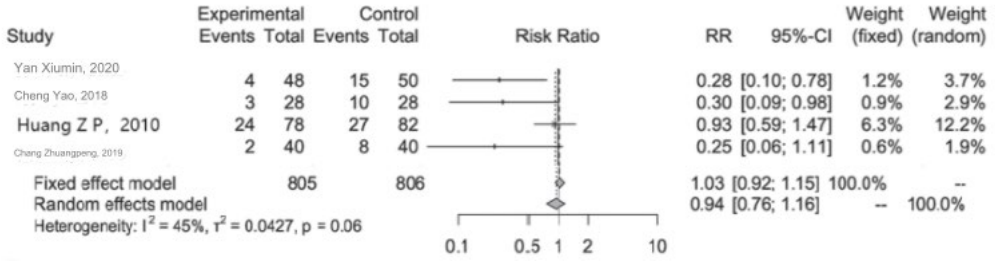


Figure 3 Effect of plumbagin on RNA methylation in PCOS

## 3 DISCUSSION

PCOS is a common endocrine and metabolic disorder syndrome in adolescent and childbearing age women, but its pathogenesis has not been clarified and its etiology is relatively complex. Western medicine is mainly based on symptomatic and supportive treatment, which can improve symptoms and delay the development of the

disease, but the efficacy is often unsatisfactory [12]. Traditional Chinese medicine believes that PCOS belongs to the categories of "amenorrhea", "infertility" and "uterine bleeding". The incidence of PCOS is mostly related to qi deficiency and phlegm stasis, because phlegm, dampness and blood are linked to each other in the uterus, and caused by micro-cysts over time. Therefore, the treatment of PCOS in traditional Chinese medicine advocates tonifying qi, promoting blood circulation and removing blood stasis [13]. In this study, 325 literatures were obtained by preliminary retrieval, and 8 literatures were finally included. Eight articles analyzed the mechanism of plumbagin in the intervention of PCOS, and the results were as follows: Plumbagin can improve the ovarian morphology and function of rats and regulate the ovarian hormone microenvironment, and its mechanism may be related to inhibiting the activity of NF-kB signaling pathway. From the results of this meta-analysis, plumbagin has a high value in PCOS, which is helpful to improve the ovarian function and morphology of PCOS rats. It can create a good microenvironment for the secretion of ovarian hormones, inhibit the NF-kB signaling pathway, and play a strong antibacterial and anti-inflammatory effect. Analysis of the reasons: Plumbagin, as one of the important traditional Chinese medicine, can produce good therapeutic effect on PCOS. Its properties are spicy, bitter, astringent and warm, and toxic. The drug can play the role of dispelling wind and dehumidifying, promoting qi and blood circulation, detoxing and detumming [14]. Modern pharmacological results show that plumbagin can exert strong anti-bacterial and anti-fungal effects, and is widely used in anti-cancer and anti-AIDS treatment. Therefore, plumbagin used in PCOS can open up a new application field in the treatment of PCOS patients and provide new intervention measures for clinical practice [15].

The occurrence and development of PCOS is often characterized by chronic low-grade inflammation, and the expression of inflammatory cytokines and inflammatory markers in PCOS is higher than that in normal people, especially for those with metabolic syndrome, often manifested as increased expression levels of interleukin-1β (IL-1β) and interleukin-8 (IL-8). In this study, 4 studies involved the

effect of plumbagin on RNA methylation in the treatment of PCOS, and the results showed that plumbagin can reverse RNA methylation in PCOS rats and improve the symptoms of PCOS. From the results, plumbagin can reverse the RNA methylation of PCOS rats, help to reduce the level of inflammatory factors in the body, thereby delaying the development of the disease. Analysis of the reason: RNA methylation can be chemically modified by the selective addition of methyl groups under the catalysis of methyltransferase. M6A methylation is reversible, involving methyltransferases, demethylases and methylation-reading proteins. RNA methylation is closely related to human development, immunity, tumor formation and metastasis, adipose differentiation and stem cell renewal. Therefore, RNA methylation can directly participate in the occurrence and development of PCOS. The application of plumbagin can reverse RNA methylation and provide a new method for the treatment of PCOS [16].

In summary, plumbagin used in PCOS is mainly used in animal experiments, which can improve the ovarian morphology and function of rats, regulate the ovarian hormone microenvironment, and thus affect RNA methylation. The mechanism may be related to the inhibition of NF-kB signaling pathway activity. However, given the quantity and quality of the included literature, further research and discussion are needed.

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REFERENCES

1. Yan Xiumin, Liu Kailiang, Gao Xinyu, et al. Study on the intervention effects of different doses of soy isoflavones on polycystic ovary syndrome rats. *Journal of Practical Obstetrics and Gynecology*, 2020, 36(8): 626-630.
2. Wang Shujuan, Yang Peihong, Jia Pingping. Effects of lifestyle intervention combined with acupuncture point embedding on infertility patients with polycystic ovary syndrome. *Journal of Practical Internal Medicine of Traditional Chinese Medicine*, 2023, 37(12): 136-139.
3. Qiao Zengfeng, Meng Junling, Jiang Yixiu. Nursing intervention of abdominal acupuncture, moxibustion combined with oral Chinese medicine for polycystic ovary syndrome. *Psychiatric Doctor*, 2016, 22(008): 25-26.
4. Wu Yifan, Huang Jing, Liu Chao, et al. Autophagy proteins and clinical data reveal the prognosis of polycystic ovary syndrome. *BMC Pregnancy and Childbirth*, 2024, 24(1): 1-11.
5. Yang Yongjun. Clinical observation of 39 cases of polycystic ovary syndrome infertility treated with integrative traditional Chinese and Western medicine. *Chinese Journal of Ethnomedicine and Ethnopharmacy*, 2018, 27(12): 128-130.
6. Huang Zhi Ping, Zhou Huan, Qu Li Hong. Maintaining a conserved methylation in plant and insect U2 small nuclear RNA through compensatory mutation by nucleotide insertion. *International Union of Biochemistry and Molecular Biology Life*, 2010, 57(10): 693-699.
7. Zhang Weiyi, Huang Guangying, Liu Jie, et al. Effects of acupuncture on infertility in polycystic ovary syndrome rats. *Chinese Journal of Integrative Medicine*, 2009, 29(11): 997-1000.
8. Cheng Yao, Shao Yunyun, Chang Zhuangpeng, et al. Intervention effects of modified Shaoyao-Gancao Decoction on polycystic ovary syndrome model rats. *Chinese Journal of Experimental Traditional Medical Formulas*, 2018, 24(6): 139-144.
9. Shen Liang, Song Chengxian, He Chuan, et al. Mechanism and function of oxidative reversal of DNA and RNA methylation. *Annual Review of Biochemistry*, 2014, 83(1): 585-614.
10. Chang Zhuangpeng, Shao Yunyun, Liu Junjin, et al. Effects of modified Shaoyao-Gancao Decoction on the NF-κB inflammatory signaling pathway in ovarian tissues of polycystic ovary syndrome rats. *Chinese Journal of Traditional Chinese Medicine and Pharmacy*, 2019, 37(9): 2295-2300, supplementary 18.

11. Yang Qi, Dong Jiaping, Xie Linlin, et al. Effects of genistein on insulin resistance in polycystic ovary syndrome rats through the adiponectin and RBP4 pathways. *Modern Food Science and Technology*, 2022, 38(9): 1-9.
12. Lin Qing, Xing Yu, Liu Yanxia, et al. Fecal microbiota transplantation experiment in letrozole-induced polycystic ovary syndrome rat model. *Modern Journal of Integrated Traditional Chinese and Western Medicine*, 2023, 32(10): 1315-1321.
13. Subbarayalu Prasad, Eedunuri Venkata Krishna, Timilsina Subash, et al. Abstract P1-04-03: Cross-talk between RNA methyltransferase and demethylase regulates breast cancer growth and progression. *Cancer Research*, 2017, 77(4 Supplement): P1-04-03-P1-04-03.
14. Jiang Xiaolin, Zhang Lide, Zhang Bing, et al. Effects of metformin combined with Xiaozhuo Decoction on inflammatory factors and sex hormone levels in polycystic ovary syndrome rats. *Journal of Beijing University of Traditional Chinese Medicine*, 2018, 041(001): 65-70.
15. Tao Xin, Ge Shuqin, Chen Jun, et al. Effects of exenatide on insulin resistance and reproductive hormones in polycystic ovary syndrome rats. *Journal of Sun Yat-sen University (Medical Science Edition)*, 2014, 35(6): 844-848, 853.
16. Xiao Hui, Liu Yanxia, Yang Yan, et al. Effects of Shenshu Zhuanghuo Decoction combined with fecal microbiota transplantation on reproductive, metabolic functions, and related inflammatory factors in letrozole-induced polycystic ovary syndrome rats. *Chinese Journal of Traditional Chinese Medicine Information*, 2020, 27(2): 33-37.

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