SIN-CHN SCIENTIFIC PRESS PTE.LTD.

Molecular & Cellular Biomechanics 2025, 22(3), 1576.
https://doi.org/10.62617/mcb1576

Article

Network pharmacology and experimental validation to investigate the effects of Fructus Psoraleae on the HPA and HPG axes of young rats

Jiayi Shan[1], Hui Qi[2], Zilun Wu[2], Ran Li[2], Yuanyuan Song[2], Chuanying Liu[2], Jiacong Xiao[2], Wenjia Zhong[3], Ying Zhang[4,*]

[1] Department of Pediatrics of Traditional Chinese Medicine, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510150, China
[2] The First Clinical Medical School, Guangzhou University of Chinese Medicine, Guangzhou 510006, China
[3] Department of Pharmacy, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510230, China
[4] Department of Pediatrics, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510230, China
*** Corresponding author:** Ying Zhang, zhangyingfly99@163.com

CITATION

Shan J, Qi H, Wu Z, et al. Network pharmacology and experimental validation to investigate the effects of Fructus Psoraleae on the HPA and HPG axes of young rats. Molecular & Cellular Biomechanics. 2025; 22(3): 1576. https://doi.org/10.62617/mcb1576

ARTICLE INFO

Received: 11 February 2025
Accepted: 18 February 2025
Available online: 27 February 2025

Abstract: To investigate the effects of Fructus Psoraleae (FP) on both the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis in juvenile rats across pre-pubertal and post-pubertal stages, as well as to explore its potential implications for biological mechanical properties, a multidisciplinary approach combining network pharmacological analysis, animal experimentation, and biomechanical assessment was employed. Fructus Psoraleae's potential pharmacological components and targets were identified via the TCMSP database. A GEO search for "precocious puberty" facilitated differential gene analysis with GEO2R. Protein interactions were examined using String, while DAVID analyzed biological processes. Molecular docking was performed using CB-Dock for validation. The effects of Fructus Psoraleae (FP) on the expression of endocrine-related proteins in the HPA and HPG axes of young rats were assessed via enzyme-linked immunosorbent assay (ELISA). Additionally, quantitative real-time polymerase chain reaction (qRT-PCR) was employed to evaluate the expression levels of relevant genes. Computational analysis revealed that FP contains 11 potential pharmacodynamic components and 19 potential targets associated with precocious puberty-related disorders. Notably, compounds such as isopsoralidin, bavachin, and psoralidin exhibited strong binding affinity to acetylcholinesterase (ACHE) targets. KEGG pathway analysis indicated their involvement in significant biological pathways, including the HIF-1 signaling pathway and AMPK signaling pathway. The ELISA results demonstrated notable differences between the FP group and the control group. During the pre-pubertal phase, the FP group exhibited significantly lower levels of corticotropin-releasing hormone (CRH) compared to the control group ($P < 0.05$). Conversely, in the post-pubertal stage, the FP group showed elevated levels of gonadotropin-releasing hormone (GnRH) relative to the control group ($P < 0.01$). From a biomechanical view, variations in hormone concentration may be linked to the regulatory effects of blood flow shear stress on the secretory activity of hypothalamic neurons. Furthermore, qRT-PCR analysis showed that estrogen receptor 1 (ESR1) expression was significantly upregulated in the FP group during the pre-pubertal stage ($P < 0.01$), while ACHE expression was notably reduced in the FP group during the post-pubertal period ($P < 0.001$). The findings suggest that Fructus Psoraleae does not exert a significant effect on the gonadal axis during the pre-pubertal phase; however, it may have the potential to activate the gonadal axis during the post-pubertal phase. Biomechanical factors may play a significant role in modulating these effects, offering fresh insights into the mechanism by which Fructus Psoraleae exerts its influence on the endocrine system.

Keywords: Fructus Psoraleae; biomechanics; network pharmacology; HPA axis; HPG axis; precocious puberty

1. Introduction

Sexual precocity is defined as a type of abnormal puberty development, characterized by the appearance of secondary sexual characteristics before 7.5 years in females and before 9 years in males [1]. The initial signs often manifest as breast enlargement in girls and testicular enlargement in boys.

The phenomenon of early sexual maturation is predominantly observed in females, with a reported ratio of girls to boys being approximately 15:20. In the United States, the estimated incidence ranges from 1 in 5000 to 1 in 10,000 girls [1,2]. Conversely, South Korea reports a prevalence of 55.9 cases per 100,000 girls, alongside a rate of 1.7 cases per 100,000 boys [3,4]. Early sexual maturation is categorized into two types: central precocious puberty (CPP) and peripheral precocious puberty (PPP) [5]. Notably, around 80% of these cases are classified as central precocious puberty, which is characterized by a dependence on gonadotropins [6].

The hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis play significant roles in the physiological development of children. The HPA axis primarily functions to manage stress responses and regulate metabolic processes, whereas the HPG axis serves as a central regulator of reproductive development and sexual maturation. A proper functionality of these two axes has an important impact on growth and development as well as physiological homeostasis in children [7]. Studies have shown that dysregulation of the HPA and HPG axes may lead to growth retardation, precocious puberty and other problems in children, so regulating the function of these two axes is important for promoting children's health [8,9].

For the treatment of CPP, long-acting gonadotropin-releasing hormone agonists (GnRHa) represent the standard of care in managing this condition. These agents work by suppressing gonadotropin secretion, thereby halting the progression of sexual maturation. However, the most frequently reported side effects include headache, hot flashes, and injection-site reactions, with weight gain emerging as a particular concern among patients. Given these considerations, exploring complementary or alternative therapeutic approaches may be warranted in certain cases.

Fructus Psoraleae (FP), a commonly employed herbal medicine in traditional Chinese medicine (TCM), is extensively used for treating osteoporosis and kidney deficiency. The seeds of this plant constitute the primary medicinal portion, which harbors diverse bioactive components including coumarins and flavonoids. These constituents exhibit significant potential in promoting bone health and enhancing immune function [10]. In addition, the potential of FP in promoting children's growth and development has been gradually valued, especially in regulating endocrine and promoting bone development, which is through the estrogen receptor (ER) pathway, one of the important pathways in regulating children's sexual development [11].

The HPA and HPG axes are critical for growth and development and endocrine function in children; however, the specific mechanisms of FP in the regulation of these axes remain unclear and systematic studies on clinical applications are scarce. In this paper, we analyse the pharmacological effects of FP and the signalling pathways it may affect by combining Network pharmacology and experimental studies, and

explore its experimental results on the hormonal effects on growth and development in young rats. It aims to provide a theoretical rationale and robust data supporting the clinical application of FP, while evaluating its therapeutic potential.

2. Materials and methods

2.1. Animals

Twenty SPF-grade SD young rats (1 week old, weighing approximately 30 ± 5 g) were obtained from the Guangdong Provincial Medical Laboratory Animal Centre. The experimental cohort included an equal number of male and female subjects, which were separately coded before random assignment into groups using numerical randomization. The animals were housed at the Guangzhou University of Chinese Medicine Laboratory Animal Centre under standard environmental conditions: temperature range 20–26 ℃, relative humidity 40%–70%, and a 12-h light/dark cycle. The young rats were housed in proximity to pregnant rats, which were provided with standard sterilized normal chow. Both pregnant and young rats had unrestricted access to food and water. This study adhered to the guidelines established by the Experimental Animal Welfare and Ethics Committee, as outlined in application form No. 20231007002 from the Experimental Animal Ethics Committee of Guangzhou University of Chinese Medicine.

2.2. Drugs

Fructus Psoraleae concentrated granules were provided by the Chinese herbal pharmacy of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (Batch No. A210A593), and each 1 g of the formulated granules was equivalent to 6.7 g of herbs.

Fructus Psoraleae concentrated granules (Batch No. A210A593) were supplied by the Chinese herbal pharmacy at the First Affiliated Hospital of Guangzhou University of Chinese Medicine. Each 1 g of granules corresponded to 6.7 g of herbs.

2.3. Reagents

AG RNAex Pro RNA Reagent (AG21101); Evo M-MLV Reverse Transcription Premix Kit (with gDNA removal reagent for qPCR) Ver.2 (AG11728); SYBR Green Pro Taq HS Premix qPCR Kit (AG11701); Rat Gonadotropin Releasing Hormone (GnRH) ELISA Kit (MEIMIAN:MM-0521R2); Rat Follicle Stimulating Hormone (FSH) ELISA Kit (MEIMIAN:MM-70867R2); Rat Luteinising Hormone (LH) ELISA Kit (MEIMIAN:MM-0624R2); Rat Testosterone (T) ELISA Kit (MEIMIAN:MM-0577R2); Rat Estradiol (E2) ELISA Kit (MEIMIAN:MM-0577R2); Rat Corticotropin-Releasing Hormone (CRH) ELISA Kit (MEIMIAN:MM-0520R2); Rat Adrenocorticotropic Hormone (ACTH) ELISA Kit (MEIMIAN:MM-0565R2); Rat Cortisol ELISA Kit (MEIMIAN:MM-0574R2).

2.4. Instruments

Analytical balance (Beijing Sartorius Balance Co., Ltd., model ALC-210.2); full-wavelength enzyme labeller (Thermo, USA, model MultiskanFC); centrifuge

(Germany eppendorf centrifuge, model centrifuge 5418); electric constant temperature water tank (Shanghai Boxun Industrial Co., Ltd. medical equipment factory, model: SSW-420-2S); clean bench (Suzhou Antai Air Technology Co., Ltd., model: LAD-LCJT1B); real-time fluorescence quantitative PCR instrument (Thermo Fisher ABI QuantStudio5, USA).

2.5. Network pharmacology studies

The active components of the traditional Chinese herbal FP were identified and their mol2 structures were retrieved from the TCMSP database (available at tcmsp-e.com/), with the screening criteria set as oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. The structural formula in mol2 format was subsequently uploaded to the PharmMapper database (accessible via http://www.lilab-ecust.cn/pharmmapper/submitfile.html), where the Targets Set parameter was configured to "Human Protein Targets Only" (version 2010, containing 2241 targets) to identify active ingredient targets with a *Z*'-score of ≥ 1.5.

The SMILES structures of the bioactive compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and subsequently uploaded to the SwissTargetPrediction platform (http://www.swisstargetprediction.ch/). We set a cutoff point at Top 15 to retrieve the biological targets associated with each active compound. The predicted target proteins were then converted into their corresponding standard gene names using the Uniprot database (www.uniprot.org). The curated target genes from both PubChem and SwissTargetPrediction databases were compiled into an Excel spreadsheet, where duplicate entries were removed through a deduplication process to derive the final list of targets for FP analysis.

We searched the GEO database of the National Center for Biotechnology Information (NCBI) (https://www. ncbi.nlm.nih.gov/geo/), and screened experimental data on human precocious puberty genes using the keyword 'precocious puberty' and differential gene analysis was performed with the GEO2R analysis tool. Two gene expression datasets, GSE7142 and GSE20872, were screened for eligibility. GSE7142 included one child with hypothalamic malformations with precocious puberty and three children with hypothalamic malformations without precocious puberty, and the microarray platform was GPL96 ([HG-U133A] Affymetrix Human Genome U133A Array). GSE208722 includes 3 cases of hypothalamic neurons with Makorin Knobbed Finger Protein 3 (MKRN3) wild-type human induced pluripotent stem cells (hiPSCs) and 3 cases of MKRN3-deficient hiPSCs, and the microarray platform is GPL20301 (Illumina HiSeq 4000).

Differential genes were screened in *R* (version 4.0.3), and the downloaded data were background corrected and normalised by the RMA algorithm, with volcano plots. Differential genes were screened with $P < 0.05$, $|\log FC| > 2$ conditions. The target genes of the diseases obtained from the above 2 datasets were imported into excel for removing duplicate values to obtain the final precocious puberty-related targets.

Draw a Wayne diagram for the intersection of FP active ingredient targets and precocious puberty targets in *R* (version 4.0.3), and submit the intersected targets to the STRING database (www.string-db.org/), with the species of 'Homo sapiens' and the confidence interval > 0.150, to construct protein-protein interaction (PPI) data.

Download its tsv format file, import it into Cytoscape 3.6.1 software, and use Network Analysis plug-in to analyse the topology of the obtained PPI network. The core target points were obtained by filtering with the median of greater than degree, closeness and betweenness as the threshold value, and the PPI network graph was plotted to represent the magnitude of the degree value of degree in terms of the node's colour shade change.

The core targets were uploaded to the DAVID database (https://david.ncifcrf.gov/summary.jsp), with 'Homo sapiens' specified as the organism and a significance threshold of $P < 0.05$ applied for filtering. The top 10 terms from Gene Ontology (GO) annotations, including Molecular Function (MF), Biological Process (BP), and Cellular Component (CC), were selected, along with the top 10 entries from Kyoto Encyclopedia of Genes and Genomes (KEGG). Network topology parameters of the active ingredients and targets were analyzed using Cytoscape software. The results were visualized in *R* as histograms and bubble charts to represent the enrichment analysis outcomes.

The three-dimensional structures (PDB entries) of the five targets with the highest degrees within the protein-protein interaction (PPI) network were retrieved from the Protein Data Bank (https://www.rcsb.org/). These structures were then docked against the bioactive components of FP utilizing the CB-Dock platform (https://cadd.labshare.cn/cb-dock2/php/index.php), a computational docking tool. Molecular docking scores were calculated, and a heatmap visualization of these interactions was generated using the *R* programming language.

2.6. Animal experiments

All rats were randomly divided into 4 groups: pre-puberty-blank group, pre-puberty-FP group, post-puberty-blank group, and post-puberty-FP group ($n = 5$ in each group). The clinical dosage of FP was 100 mg/kg, and the equivalent dose for rats was converted according to body surface area. After 3 days of acclimatization, both blank groups were orally administered with 10 mL/kg of PBS solution, while both FP groups received 10 mL/kg of FP granules based on the equivalent dose calculated according to daily body weight. The gavage was performed once a day for all groups. After 7 days of gavage (23 days of age), the pre-puberty 2 groups were sacrificed. The post-puberty 2 groups continued the gavage intervention for 7 days and were sacrificed after 14 days of gavage (30 days of age).

Throughout the experimental duration, subjects were monitored for alterations in mental status, while body weight variations across all groups were measured and documented on a daily basis.

Eye blood sampling was performed at the time of sacrifice in the pre-puberty 2 groups and post-puberty 2 groups and serum was separated. Serum levels of GnRH, FSH, LH, T, E2, CRH, ACTH and CORT were measured according to the instructions of the ELISA kit.

Based on the results of Network pharmacology, we chose ESR1, ESR2 and ACHE to explore the mechanism of FP's effect on sexual development. Pre-puberty and post-puberty young rats' ovaries or testes were selected. Total RNA from ovaries or testes was extracted using TRIzol and phenol-chloroform phase separation according to the manufacturer's instructions, followed by the use of Evo M-MLV

Reverse Transcription Premixed Kit and SYBR Green Pro Taq HS Premixed qPCR Kit. Primer sequences for qRT-PCR are listed in **Table 1**.

Table 1. Real-time polymerase chain reaction primers.

PubChem CID	Component Name	Molecular Formula	Molecular Weight (g/mol)
10658	angelicin	C11H6O3	186.16
5468522	bakuchiol	C18H24O	256.399
6450879	bavachalcone	C20H20O4	324.4
14236566	bavachin	C20H20O4	324.4
5321800	bavachromene	C20H18O4	322.4
5281255	isobavachalcone	C20H20O4	324.4
193679	isobavachin	C20H20O4	324.4
5281806	psoralidin	C20H16O5	336.3
5280794	stigmasterol	C29H48O	412.7
14630492	sophoraccoumestan A	C20H14O5	334.3
12304285	isopsoralidin	C20H16O5	336.3

2.7. Statistical analysis

The experimental results were statistically analyzed using GraphPad Prism software. Single-factor ANOVA was employed to assess differences in experimental data across groups. Significance was defined as a *P*-value less than 0.05.

3. Results

3.1. Network pharmacology

Eleven potential active ingredients of FP were obtained from TCMSP database search, and the results were shown in **Table 1**, with 191 active compound targets. The up- and down-regulated genes of GSE7142H and GSE208722 were screened from GEO database, and the volcano diagram is shown in **Figure 1**. A total of 1172 disease targets were retrieved, and the intersection of drug component genes and disease genes was obtained in *R*. 19 intersected targets were obtained, and the Wayne diagrams are shown in **Figure 1** and **Table 2**. A network diagram of drug-component-target-disease interactions was drawn using Cytoscape software, see **Figure 1**.

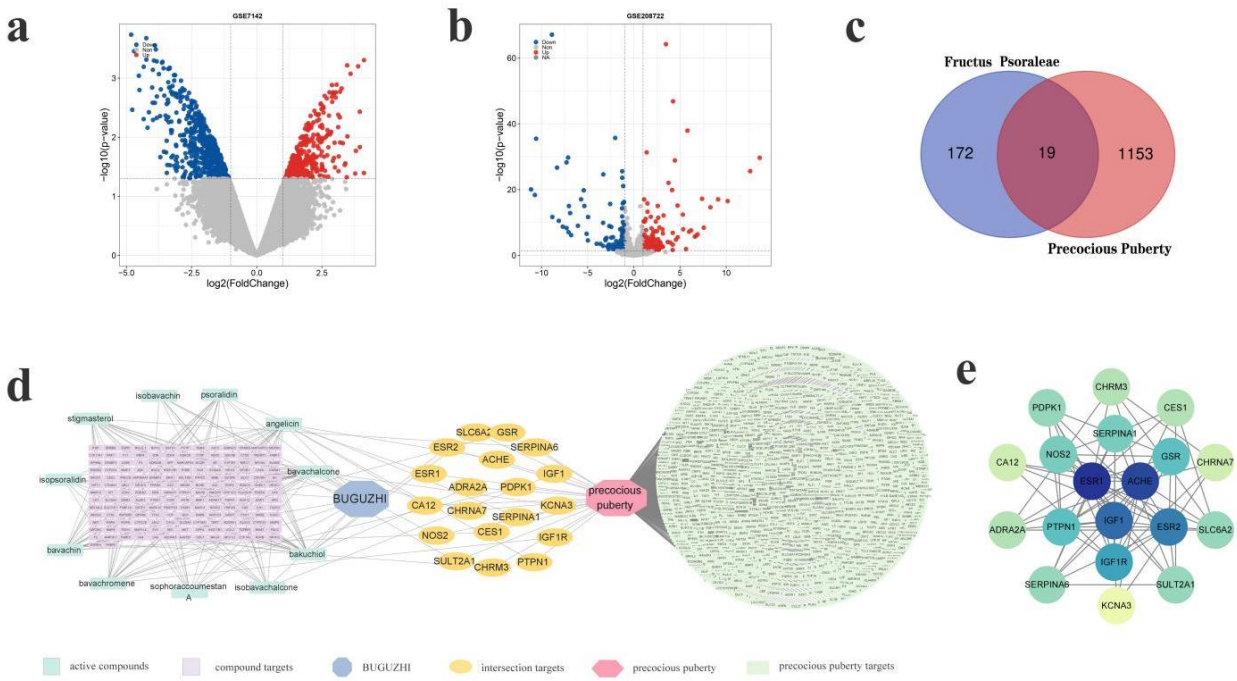

Figure 1. (a) Volcano plot of differentially gene expression in precocious puberty (GSE7142H database); **(b)** Volcano plot of differentially gene expression in precocious puberty (GSE208722 database); **(c)** The veen diagram about the target of precocious puberty and the target of Fructus Psoraleae; **(d)** The drug-target-disease network; **(e)** PPI network construction.

Table 2. Characteristics of the 19 hub gene.

Gene	Description	Degree	Betweenness centrality	Closeness centrality
ESR1	Estrogen Receptor 1	16	0.17	0.90
ACHE	Acetylcholinesterase	15	0.19	0.86
IGF1	Insulin Like Growth Factor 1	13	0.10	0.78
ESR2	Estrogen Receptor 2	12	0.06	0.75
IGF1R	Insulin Like Growth Factor 1 Receptor	10	0.05	0.69
GSR	Glutathione-Disulfide Reductase	8	0.00	0.64
PTPN1	Protein Tyrosine Phosphatase Non-Receptor Type 1	8	0.00	0.64
NOS2	Nitric Oxide Synthase 2	7	0.01	0.62
SERPINA1	Serpin Family A Member 1	7	0.02	0.62
SLC6A2	Solute Carrier Family 6 Member 2	6	0.01	0.60
SERPINA6	Serpin Family A Member 6	6	0.00	0.60
SULT2A1	Sulfotransferase Family 2A Member 1	6	0.01	0.58
PDPK1	3-Phosphoinositide Dependent Protein Kinase 1	6	0.00	0.60
CHRM3	Cholinergic Receptor Muscarinic 3	5	0.01	0.58
ADRA2A	Adrenoceptor Alpha 2A	5	0.00	0.58
CES1	Carboxylesterase 1	5	0.01	0.58
CA12	Carbonic Anhydrase 12	4	0.00	0.56
CHRNA7	Cholinergic Receptor Nicotinic Alpha 7 Subunit	4	0.01	0.55
KCNA3	Potassium Voltage-Gated Channel Subfamily A Member 3	3	0.00	0.55

3.2. GO function and KEGG pathway enrichment analysis

The 19 core targets in **Table 2** were functionally annotated by DAVID, and the *P* values were sorted from smallest to largest, and the top 10 entries of each of the biological process (BP), cellular component (CC) and molecular function (MF) in the GO function annotations were selected, and the GO bubble diagrams and histograms were drawn by *R* for visual analysis, and the results are shown in **Figure 2**. Among them, 37 pathways are related to biological processes (BP), and the top 10 mainly include acetylcholine receptor signaling pathway, acetylcholine receptor signaling pathway, insulin-like growth factor receptor signaling pathway, positive regulation of MAPK cascade, cellular response to estradiol stimulus, and insulin receptor signaling pathway, intracellular steroid hormone receptor signaling pathway. 9 components related to cellular components (CC), mainly including plasma membrane, presynaptic membrane, postsynaptic membrane, basolateral plasma membrane, integrative component of plasma membrane. There are 16 molecular functions (MF), mainly including acetylcholine binding, insulin receptor binding, steroid binding, estrogen receptor activity, estrogen response element binding, steroid hormone receptor activity and others.

A total of 15 signaling pathways were identified and analyzed through KEGG, with the results presented in **Figure 2**. These include Endocrine Resistance (Pathway), HIF-1 Signaling Pathway, Cholinergic Synapse, AMPK Signaling Pathway, FoxO Signaling Pathway, mTOR Signaling Pathway, Aldosterone-regulated Sodium Reabsorption, and Ovarian Steroidogenesis.

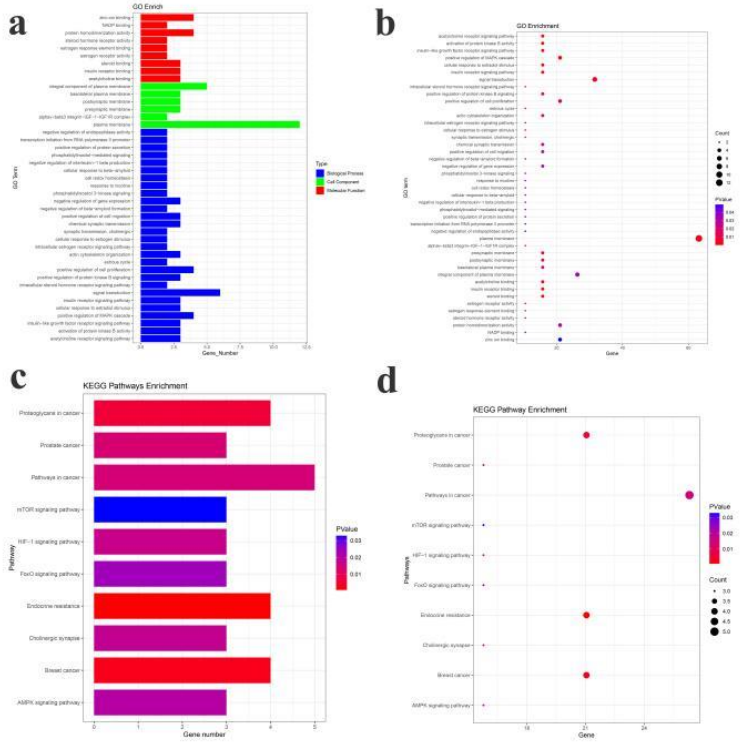

Figure 2. (a) GO enrichment analysis (bubble diagram); **(b)** GO enrichment analysis(histogram); **(c)** KEGG enrichment analysis (bubble diagram); **(d)** KEGG enrichment analysis (histogram).

3.3. Docking results

The active components of FP in **Table 1** were docked molecularly with the top 5 targets in terms of degree value in the PPI network diagram in **Figure 3**, and the binding strength of the target and the active compounds was evaluated according to the value of Vina Score, the lower the value of Vina Score, the more stable the molecular conformation was, and the results of the docking were plotted as a heatmap, see **Figure 3**. Most of the molecular docking results had a Vina Score < −7.0 indicating that all the 11 active compounds in FP showed better binding ability to the top 5 ranked targets. Among them, the ACHE showed the best binding to isopsoralidin, bavachin, psoralidin, bavachromene, and sophorus Psoraleae coumarin estane A.

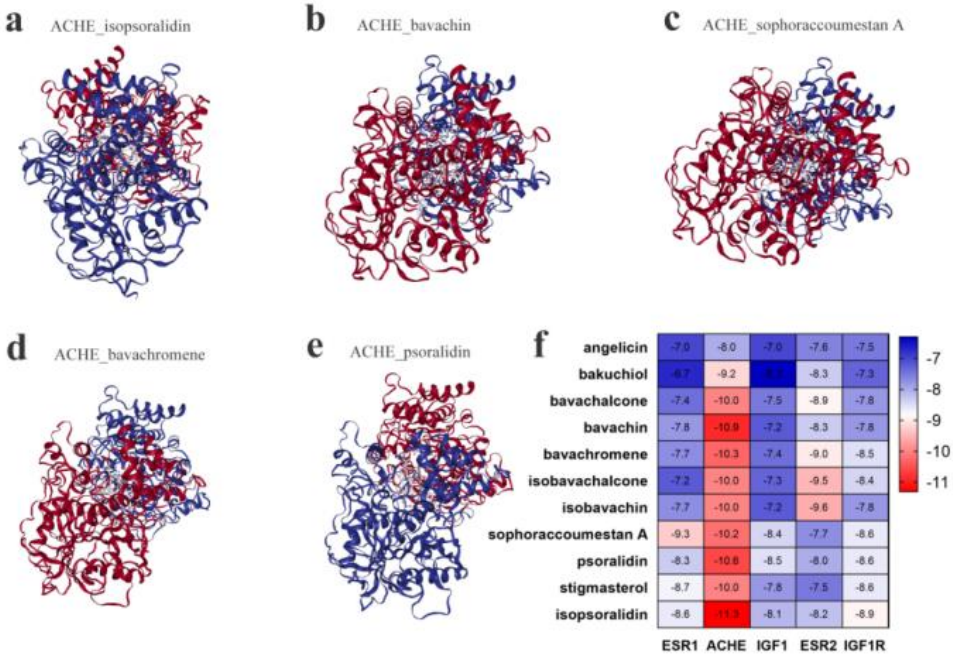

Figure 3. Five hub genes and the 11 significant Molecular Docking: **(a)** ACHE, isopsoralidin, −11.3 kcal/mol; **(b)** ACHE, bavachin, −10.9 kcal/mol; **(c)** ACHE, sophoraccoumestan A, −10.2 kcal/mol; **(d)** ACHE, bavachromene, −10.3 kcal/mol; **(e)** ACHE, psoralidin, −10.6 kcal/mol; **(f)** Heatmaps of the docking scores of hub genes combined with bioactive compound of Fructus Psoraleae.

3.4. Body weight

During the pre-puberty period, no statistically significant differences in body weight were observed between FP groups and control groups. However, during the post-puberty period, the FP groups exhibited slower weight gain compared to control groups, although no statistically significant difference was noted at the endpoint (**Figure 4**). In addition, the rats in the four groups had good mental status, similar appearance and development, no irritability or agitation, and no death during the dosing period.

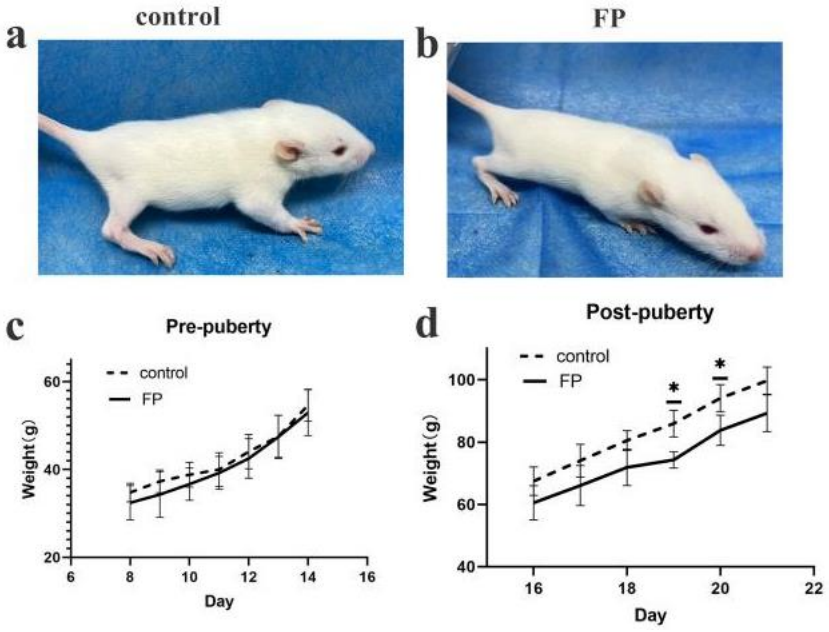

Figure 4. (a) Appearance of rats in control group after oral administration of PBS; **(b)** Appearance of rats in control group after oral administration of Fructus Psoraleae; **(c)** Plot of weight change of pre-puberty; **(d)** Plot of weight change of post-puberty.

3.5. ELISA results

For pre-puberty, CRH expression in the FP groups was lower than that in the control groups ($P < 0.05$), suggesting that oral administration of FP may suppress pre-puberty CRH expression. No statistical significance was observed for the remaining indicators, see **Figure 5**. For post-puberty, GnRH expression was higher in the FP groups than in the control groups ($P < 0.01$), suggesting that oral FP may promote post-puberty GnRH expression. The remaining indicators did not show statistical significance, see **Figure 5**.

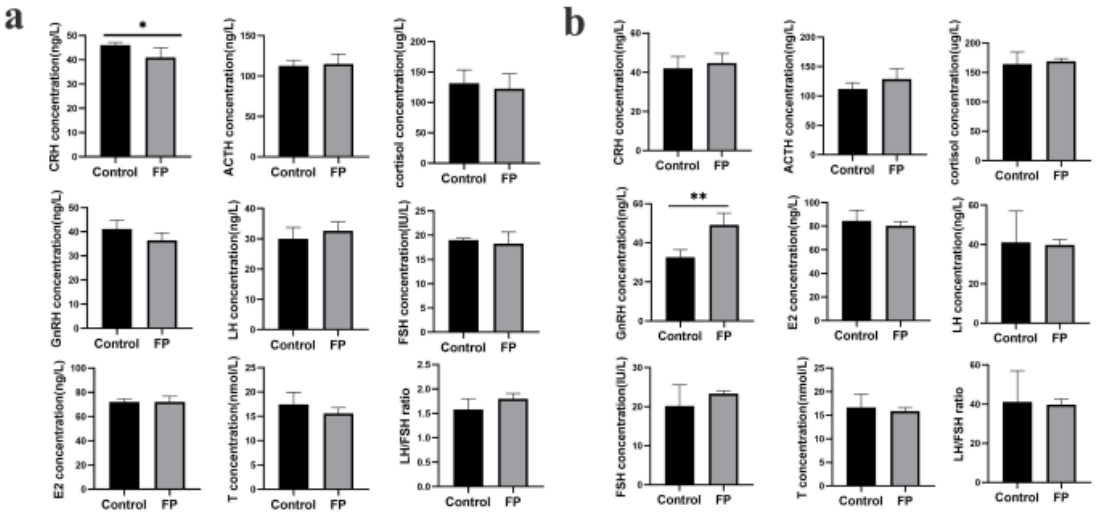

Figure 5. Effect of FP on hormone levels of HPA and HPG axis: **(a)** hormone levels of pre-puberty; **(b)** hormone levels of post-puberty.

*$P < 0.05$; ** $P < 0.01$.

3.6. qRT-PCR measurement of ESR1, ESR2, ACHE

We used qRT-PCR to measure the effect of FP group and control group on the mRNA expression of ESR1, ESR2, and ACHE. The results showed that before puberty，the mRNA expression levels of ESR1 were higher in the FP group than in the control group ($P < 0.01$). After puberty, the mRNA expression of ACHE in FP group was significantly decreased compared with that in the control group ($P < 0.001$), see **Figure 6**.

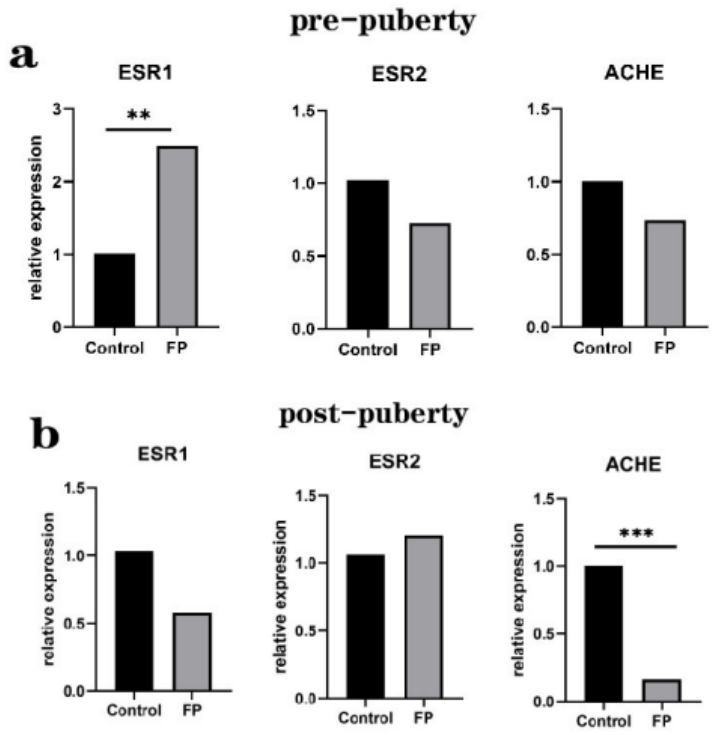

Figure 6. Effect of FP on ESR1, ESR2 and ACHE mRNA: **(a)** represents the relative mRNA expression of pre-puberty; **(b)** represents the relative mRNA expression of post-puberty.

$^{**}P < 0.01$; $^{***}P < 0.001$.

4. Discussion

Precocious puberty in children is a matter of great concern for paediatricians worldwide, as it not only affects the child's body shape, but also imposes a burden on the child's present and future psychological well-being. The mechanism of action of central precocious puberty (CPP), which is the largest part of the classification, is the early initiation of the HPG axis [1], and to a lesser extent the HPA axis also plays an influential role [12].

Current diagnostic approaches emphasize hormonal evaluation and imaging modalities to identify and monitor the progression of precocious puberty [13]. In terms of treatment, long-acting gonadotropin-releasing hormone agonists (GnRHa) remain the standard therapeutic option for CPP, as they effectively suppress gonadotropin secretion [14]. Notably, the most frequently reported side effects associated with GnRHa therapy include headaches, hot flashes, and injection-site reactions. Among potential challenges, weight gain represents a notable concern during treatment [15].

Therefore it is essential to seek some complementary or replacement therapies. Fructus Psoraleae (FP), as a traditional Chinese herbal medicine, the main components of which include psoralen, coumarin and flavonoids, etc. Traditionally, it has been used to promote bone growth and improve sexual function, and it is often used in childhood precocious puberty disorders, but the mechanism of its action is still not clear enough. Therefore, this paper explores the effects of Fructus Psoraleae on the HPA and HPG axes in young rats through Network pharmacology and experiments to promote its application in modern medicine.

In this paper, we identified 11 potential active components of Fructus Psoraleae through Network pharmacology, including angelicin, bakuchiol, isobavachin, psoralidin, stigmasterol, isopsoralidin and so on. Although there are no more studies to confirm the effects of the above active ingredients in humans or animals with precocious puberty disease, some studies have been done to confirm that some of the active ingredients have hormone-regulating effects. stigmasterol has good estrogenic activity [16], and its oxidation products have interfered with endogenous estrogenic effects [17], and in vitro experiments have also confirmed the estrogen-like effects of stigmasterol [18]. bakuchiol can regulate estrogen signalling by down-regulation of matrix aromatase and up-regulation of epithelial estrogen receptor β (ERβ) [19]. In in vitro experiments, the affinity of bakuchiol for estrogen receptor alpha (ERα) was higher than that for ERβ, and the application of bakuchiol to treat rats with ovarian removal model increased their plasma levels of estradiol [20]. Angelicin increases the expression of Erα [21], which promotes osteogenic differentiation, longitudinal bone growth, and positively regulates estrogen-deficient osteoporosis. isobavachin effectively regulates ER targets by binding to both ERα and ERβ isoforms of the estrogen receptor (ER) [22]. isobavachalcone has a high affinity for ERα and can delay bone loss in de-ovulated osteoporotic mice by increasing plasma estradiol levels [23]. In summary, it can be seen that most of the active ingredients of Fructus Psoraleae have estrogen-like physiological effects.

Through KEGG pathway enrichment analysis, we identified that Fructus Psoraleae may exert its effects on precocious puberty via several signaling pathways, including HIF-1, AMPK, FoxO, and mTOR signaling pathways.

HIF-1 exerts significant influence on endocrine system regulation, playing a key role in modulating glandular function and hormone secretion across multiple endocrine organs. Research has demonstrated that HIF-1 has a critical impact on the functionality of endocrine glands, such as the thyroid and ovarian glands, by affecting hormone synthesis and release while also participating in the regulatory mechanisms of reproductive physiology and metabolic processes [24,25]. HIF-1 further enhances its role in endocrine regulation by interacting with other signalling pathways, such as the mTOR and VEGF signalling pathways, and shows promise as a potential therapeutic target [26]. Chen et al. propose that CPP in TCM is influenced by an imbalance between kidney Yin and Yang. To investigate this, they employed a network pharmacological approach to analyze the therapeutic effects of Dabuyin Pill on CPP. Their study revealed that the underlying molecular mechanisms were significantly enriched in the HIF-1 signaling pathway [27]. This research is expected to provide novel insights and therapeutic strategies for the treatment of precocious

puberty and related disorders by elucidating the endocrine system's response to FP through its modulation of the HIF-1 pathway.

AMP-activated protein kinase (AMPK) is an important energy-sensing enzyme that plays a significant role in regulating cellular metabolism. Activation of AMPK stimulates fatty acid oxidation, suppresses lipid synthesis, and enhances glucose uptake and utilization, thereby maintaining cellular energy balance [28]. In addition, AMPK plays an important role in the regulation of cell growth, proliferation and apoptosis, especially while dealing with nutrient deficiencies and metabolic stress, AMPK maintains cellular homeostasis by regulating downstream signalling pathways (e.g., mTOR and SIRT1) [29]. Recent studies have also highlighted that the role of AMPK in endocrine regulation is becoming better understood and may be linked to the mechanisms underlying precocious puberty, suggesting that investigating the function of the AMPK signaling pathway in this condition could offer novel insights into potential therapeutic strategies [30]. The active ingredients in Fructus Psoraleae, such as isobavachalcone and other flavonoids, have been shown to be effective in activating the AMPK signalling pathway. Studies on FoxO signaling pathway and precocious puberty have been less frequently reported. Studies on FoxO signaling pathway have mainly focused on bone development and cancer, but in animal disciplines, some studies have revealed that FoxO signaling pathway can regulate the juvenile hormone of silkworm mutants to control insect development and delay insect growth [31].

After molecular docking, we found that the main gene responsible for Fructus Psoraleae's action on precocious puberty is ACHE. The ACHE gene encodes acetylcholinesterase (ACHE), whose main function is to catalyse the hydrolysis of the neurotransmitter acetylcholine, thereby terminating neural signalling. The ACHE gene is mapped to human chromosome 7q22.1 and features an intricate structure with multiple exons and introns; its transcript produces several homologues through diverse splicing patterns [32]. Studies have demonstrated that acetylcholine modulates the function of the HPG axis by binding to its receptor, thereby influencing the secretion of sex hormones. The expression level of the ACHE gene directly impacts acetylcholine metabolism, which in turn affects the synthesis and release of sex hormones [33]. For instance, experimental findings in specific animal models have revealed that inhibiting ACHE activity can result in elevated levels of sex hormones [34]. In addition, functional changes in the ACHE gene may further affect the onset of precocious puberty by influencing the development and function of reproductive organs [35].

And our animal experiments can also confirm the above points. We measured changes in HPG axis hormones during the pre-puberty and post-puberty periods by ELISA, and we found that oral administration of Fructus Psoraleae increased GnRH levels in rats during the post-puberty period. Meanwhile, by qRT-PCR measurements, we found that ACHE gene expression is reduced in the FP group during the post-puberty period. Therefore, we hypothesised that after oral administration of Fructus Psoraleae, the ACHE gene is suppressed at the post-puberty stage, which leads to the elevation of GnRH. Interestingly, however, no changes in the HPG axis were detected in the FP group at the pre-puberty stage, which potentially suggests that the gonadal effects of Fructus Psoraleae on young rats are related to whether the animals are

developing or not. Oral administration of Fructus Psoraleae at the immature stage did not affect the HPG axis.

Furthermore, our findings indicated that CRH expression during pre-puberty was significantly lower in the FP group compared to controls. This observation possibly reflects Fructus Psoraleae's role in modulating the HPA axis activity and mitigating stress responses. The role of CRH in precocious puberty has been gradually attracting attention, and several studies have shown that CRH may affect the occurrence of precocious puberty through multiple mechanisms. Some studies have shown that Fructus Psoraleae may inhibit the overproduction of CRH by regulating the release of neurotransmitters, and has an antidepressant effect in mice [36]. In addition, early life adversity accelerates the hypothalamic drive for sexual maturation, leading to the onset of precocious puberty, which may be associated with abnormal secretion of CRH [37]. In animal experiments, CRH overexpression was found to be associated with disturbances in the estrous cycle and LH (luteinising hormone) dynamics in female mice, suggesting that CRH may influence the timing of sexual maturation by modulating the neuroendocrine activity of the hypothalamus [38]. In addition, CRH interacts with other hormones such as growth hormone and sex hormones. CRH has been found to inhibit the secretion of growth hormone [39], whereas the levels of sex hormones are also affected by CRH under stress, which may lead to changes in reproductive function [40]. For example, it has been shown that CRH leads to a significant reduction in progesterone levels in a dose-dependent manner [41]. Therefore, in our study, we found that the expression of CRH during pre-puberty was reduced in the FP group compared to the control group, although there was no significant change in the HPG axis, which may suggest that Fructus Psoraleae can down-regulate the HPA axis, and there is a potential to promote the increase of sex hormones.

However, our study has the following shortcomings. Firstly, we did not differentiate the rats by sex during our experiments. According to previous studies, the maturation time of gonads and hormone secretion are different in animals of different sexes [42]. The current results of our study, which included both female and male rats, did not distinguish in detail the different effects of Fructus Psoraleae on female and male rats, which will be addressed in our next experiments. Secondly, we uncovered several potential mechanistic pathways through KEGG, but this experiment did not validate the relevant proteins on the pathways, which requires further research.

FP is widely used in traditional medicine, and with in-depth research into its bioactive components, its potential in regulating the HPA axis and HPG expression is starting to gain recognition. Future research should investigate several critical areas to enhance the clinical application of FP in regulating HPG expression during puberty and modulating HPA axis function. A foremost priority is expanding study sample sizes and implementing multi-center trials, which are essential for improving both the reliability and generalizability of research outcomes. Large-scale clinical trials can give us a clearer picture of how effective and safe FP is. Second, we should look into how FP works, especially the differences between genders and age groups. We can do this through stratified analysis and studying the mechanisms to reveal the specific pathways through which FP regulates the HPA axis. Furthermore, future studies should also focus on assessing the long-term safety of FP. By conducting long-term

follow-up studies, we can learn about the long-term effects of FP on kids before puberty, particularly regarding potential risks to reproductive health and endocrine function. Finally, with the advancement of modern technology, research on FP that uses big data and AI will also be a key focus. By analyzing a large amount of clinical data, we can figure out the best uses and treatment plans for FP across different groups, thus achieving personalized treatment.

5. Conclusion

Our study found that Fructus Psoraleae has 11 potential pharmacological components and 19 potential targets to act on precocious puberty diseases, ACHE is the key gene, HIF-1 signaling pathway, AMPK signaling pathway and so on are the potential signalling pathways. The ELISA test of animal experiments found that the Chinese herb Fructus Psoraleae did not affect the gonadal axis significantly in pre-puberty of normal development, but for normal post-puberty development there exists a potential possibility of initiating the gonadal axis, which indicating that Fructus Psoraleae affects gonadal secretion in post-puberty development.

Author contributions: Conceptualization, JS and ZW; methodology, WZ, JX, HQ and ZW; software, YS; validation, RL; formal analysis, HQ and ZW; investigation, RL and CL; resources, YZ; data curation, HQ and ZW; writing—original draft preparation, HQ; writing—review and editing, YZ; visualization, HQ and ZW; supervision, YS; project administration, YZ; funding acquisition, YZ. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Traditional Chinese Medicine Bureau of Guangdong Provincial (grant number: 20221156).

Ethical approval: The studies were conducted in accordance with the Declaration of Helsinki, involving animal participants were reviewed and approved by Experimental Animal Ethics Committee of Guangzhou University of Chinese Medicine. The ethics approval number is 20231007002.

Conflict of interest: The authors declare no conflict of interest.

References

1. Latronico AC, Brito VN, Carel JC. Causes, diagnosis, and treatment of central precocious puberty. Lancet Diabetes Endocrinol. 2016; 4(3): 265-74. doi: 10.1016/S2213-8587(15)00380-0
2. Partsch CJ. Pathogenesis and epidemiology of precocious puberty. Effects of exogenous oestrogens. Human Reproduction Update. 2001; 7(3): 292-302. doi: 10.1093/humupd/7.3.292
3. Kim SH, Huh K, Won S, et al. A Significant Increase in the Incidence of Central Precocious Puberty among Korean Girls from 2004 to 2010. PLOS ONE. 2015; 10(11): e0141844. doi: 10.1371/journal.pone.0141844
4. Soriano-Guillén L, Corripio R, Labarta JI, et al. Central Precocious Puberty in Children Living in Spain: Incidence, Prevalence, and Influence of Adoption and Immigration. The Journal of Clinical Endocrinology & Metabolism. 2010; 95(9): 4305-4313. doi: 10.1210/jc.2010-1025
5. Brito VN, Spinola-Castro AM, Kochi C, et al. Central precocious puberty: revisiting the diagnosis and therapeutic management. Archives of Endocrinology and Metabolism. 2016; 60(2): 163-172. doi: 10.1590/2359-3997000000144

6. Parent AS, Teilmann G, Juul A, et al. The Timing of Normal Puberty and the Age Limits of Sexual Precocity: Variations around the World, Secular Trends, and Changes after Migration. Endocrine Reviews. 2003; 24(5): 668-693. doi: 10.1210/er.2002-0019
7. Tong X, Yang Y, Gong S, et al. Data Mining and Network Pharmacology Analysis of Kidney-Tonifying Herbs on the Treatment of Renal Osteodystrophy Based on the Theory of "Kidney Governing Bones" in Traditional Chinese Medicine. Evidence-Based Complementary and Alternative Medicine. 2022; 2022: 1-13. doi: 10.1155/2022/1116923
8. Cutia CA, Christian-Hinman CA. Mechanisms linking neurological disorders with reproductive endocrine dysfunction: Insights from epilepsy research. Frontiers in Neuroendocrinology. 2023; 71: 101084. doi: 10.1016/j.yfrne.2023.101084
9. Allen JO, Mezuk B, Byrd DR, et al. Mechanisms of Cardiometabolic Health Outcomes and Disparities: What Characteristics of Chronic Stressors are Linked to HPA-Axis Dysregulation?. Journal of Aging and Health. 2022; 34(3): 448-459. doi: 10.1177/08982643221085903
10. Chen L, Chen S, Sun P, et al. Psoralea corylifolia L.: a comprehensive review of its botany, traditional uses, phytochemistry, pharmacology, toxicology, quality control and pharmacokinetics. Chinese Medicine. 2023; 18(1). doi: 10.1186/s13020-022-00704-6
11. Luo Y, Liu Q, Lei X, et al. Association of estrogen receptor gene polymorphisms with human precocious puberty: a systematic review and meta-analysis. Gynecological Endocrinology. 2015; 31(7): 516-521. doi: 10.3109/09513590.2015.1031102
12. Yang L, Tang K, Qi Y, et al. Potential metabolic mechanism of girls' central precocious puberty: a network analysis on urine metabonomics data. BMC Systems Biology. 2012; 6(S3). doi: 10.1186/1752-0509-6-s3-s19
13. Cheuiche AV, da Silveira LG, de Paula LCP, et al. Diagnosis and management of precocious sexual maturation: an updated review. European Journal of Pediatrics. 2021; 180(10): 3073-3087. doi: 10.1007/s00431-021-04022-1
14. Belchetz PE, Plant TM, Nakai Y, et al. Hypophysial Responses to Continuous and Intermittent Delivery of Hypothalamic Gonadotropin-Releasing Hormone. Science. 1978; 202(4368): 631-633. doi: 10.1126/science.100883
15. Yang WJ, Ko KH, Lee KH, et al. The different effects of gonadotropin-releasing hormone agonist therapy on body mass index and growth between normal-weight and overweight girls with central precocious puberty. Annals of Pediatric Endocrinology & Metabolism. 2017; 22(1): 49. doi: 10.6065/apem.2017.22.1.49
16. Boldrin PK, Resende FA, Höhne APO, et al. Estrogenic and mutagenic activities of Crotalaria pallida measured by recombinant yeast assay and Ames test. BMC Complementary and Alternative Medicine. 2013; 13(1). doi: 10.1186/1472-6882-13-216
17. Newill H, Loske R, Wagner J, et al. Oxidation products of stigmasterol interfere with the action of the female sex hormone 17β-estradiol in cultured human breast and endometrium cell lines. Molecular Nutrition & Food Research. 2007; 51(7): 888-898. doi: 10.1002/mnfr.200700025
18. Sriraman S, Ramanujam GM, Ramasamy M, et al. Identification of beta-sitosterol and stigmasterol in Bambusa bambos (L.) Voss leaf extract using HPLC and its estrogenic effect in vitro. Journal of Pharmaceutical and Biomedical Analysis. 2015; 115: 55-61. doi: 10.1016/j.jpba.2015.06.024
19. Miao L, Jiao C, Shao R, et al. Bakuchiol suppresses oestrogen/testosterone-induced Benign Prostatic Hyperplasia development through up-regulation of epithelial estrogen receptor β and down-regulation of stromal aromatase. Toxicology and Applied Pharmacology. 2019; 381: 114637. doi: 10.1016/j.taap.2019.114637
20. Lim SH, Ha TY, Kim SR, et al. Ethanol extract ofPsoralea corylifoliaL. and its main constituent, bakuchiol, reduce bone loss in ovariectomised Sprague–Dawley rats. British Journal of Nutrition. 2008; 101(7): 1031-1039. doi: 10.1017/s0007114508066750
21. Ge L, Cui Y, Liu B, et al. ERα and Wnt/β-catenin signaling pathways are involved in angelicin-dependent promotion of osteogenesis. Molecular Medicine Reports. 2019. doi: 10.3892/mmr.2019.9999
22. Park JW, Kim DH, Ahn HN, et al. Activation of Estrogen Receptor by Bavachin from Psoralea corylifolia. Biomolecules and Therapeutics. 2012; 20(2): 183-188. doi: 10.4062/biomolther.2012.20.2.183
23. Wang X, Ji Q, Hu W, et al. Isobavachalcone prevents osteoporosis by suppressing activation of ERK and NF-κB pathways and M1 polarization of macrophages. International Immunopharmacology. 2021; 94: 107370. doi: 10.1016/j.intimp.2021.107370
24. Wang Y, Wang Y, Chen Y, et al. HDAC5 inhibits ovarian angiogenesis in dehydroepiandrosterone-induced mouse model of polycystic ovary syndrome. Folia Histochemica et Cytobiologica. 2022; 60(3): 260-270. doi: 10.5603/fhc.a2022.0024

25. Heydarzadeh S, Moshtaghie AA, Daneshpoor M, et al. Regulators of glucose uptake in thyroid cancer cell lines. Cell Communication and Signaling. 2020; 18(1). doi: 10.1186/s12964-020-00586-x
26. Samaan E, Ramadan NM, Abdulaziz HMM, et al. DPP-4i versus SGLT2i as modulators of PHD3/HIF-2α pathway in the diabetic kidney. Biomedicine & Pharmacotherapy. 2023; 167: 115629. doi: 10.1016/j.biopha.2023.115629
27. Chen X, Zheng M, Fei X, et al. Analysis of the efficacy of Dabuyin pill combined with gonadotropin-releasing hormone analogue in the treatment of central precocious puberty girls based on network pharmacology. Translational Pediatrics. 2023; 12(3): 364-374. doi: 10.21037/tp-23-111
28. Lu G, Wu Z, Shang J, et al. The effects of metformin on autophagy. Biomedicine & Pharmacotherapy. 2021; 137: 111286. doi: 10.1016/j.biopha.2021.111286
29. Guo P, Zeng M, Liu M, et al. Isolation of Calenduloside E from Achyranthes bidentata Blume and its effects on LPS/D-GalN-induced acute liver injury in mice by regulating the AMPK-SIRT3 signaling pathway. Phytomedicine. 2024; 125: 155353. doi: 10.1016/j.phymed.2024.155353
30. Baumgartner C, Yadav AK, Chefetz I. AMPK-like proteins and their function in female reproduction and gynecologic cancer. Regulation of Downstream Targets. 2023. doi: 10.1016/bs.apcsb.2022.11.016
31. Zeng B, Huang Y, Xu J, et al. The FOXO transcription factor controls insect growth and development by regulating juvenile hormone degradation in the silkworm, Bombyx mori. Journal of Biological Chemistry. 2017; 292(28): 11659-11669. doi: 10.1074/jbc.m117.777797
32. Xu ML, Luk WKW, Liu EYL, et al. Differentiation of erythroblast requires the dimeric form of acetylcholinesterase: Interference with erythropoietin receptor. Chemico-Biological Interactions. 2019; 308: 317-322. doi: 10.1016/j.cbi.2019.06.006
33. Vastagh C, Farkas I, Csillag V, et al. Cholinergic control of GnRH neuron physiology and luteinizing hormone secretion in male mice: involvement of ACh/GABA co-transmission. The Journal of Neuroscience. 2024. doi: 10.1523/jneurosci.1780-23.2024
34. Leonard ST, Hearn JK, Catling AD, et al. Gonadal hormones modulate the potency of the disruptive effects of donepezil in male rats responding under a nonspatial operant learning and performance task. Behavioural Pharmacology. 2010; 21(2): 121-134. doi: 10.1097/fbp.0b013e328337be3a
35. Klüver N, Yang L, Busch W, et al. Transcriptional Response of Zebrafish Embryos Exposed to Neurotoxic Compounds Reveals a Muscle Activity Dependent hspb11 Expression. PLoS ONE. 2011; 6(12): e29063. doi: 10.1371/journal.pone.0029063
36. Yi LT, Li YC, Pan Y, et al. Antidepressant-like effects of psoralidin isolated from the seeds of Psoralea Corylifolia in the forced swimming test in mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2008; 32(2): 510-519. doi: 10.1016/j.pnpbp.2007.10.005
37. Granata L, Fanikos M, Brenhouse HC. Early life adversity accelerates hypothalamic drive of pubertal timing in female rats with associated enhanced acoustic startle. Hormones and Behavior. 2024; 159: 105478. doi: 10.1016/j.yhbeh.2024.105478
38. Yu J, Li XF, Tsaneva-Atanasova K, et al. Chemogenetic activation of PVN CRH neurons disrupts the estrous cycle and LH dynamics in female mice. Frontiers in Endocrinology. 2024; 14. doi: 10.3389/fendo.2023.1322662
39. Ghizzoni L, Vottero A, Street ME, et al. Dose-dependent inhibition of growth hormone (GH)-releasing hormone-induced GH release by corticotropin-releasing hormone in prepubertal children. The Journal of Clinical Endocrinology & Metabolism. 1996; 81(4): 1397-1400. doi: 10.1210/jcem.81.4.8636340
40. Wang YT, Wu QH, Chen L, et al. Effects of sub-chronic exposure to microcystin-LR on the endocrine system of male rats. Science of The Total Environment. 2024; 906: 166839. doi: 10.1016/j.scitotenv.2023.166839
41. Yang R, You X, Tang X, et al. Corticotropin-releasing hormone inhibits progesterone production in cultured human placental trophoblasts. Journal of Molecular Endocrinology. 2006; 37(3): 533-540. doi: 10.1677/jme.1.02119
42. Kauffman AS, Navarro VM, Kim J, et al. Sex differences in the regulation of Kiss1/NKB neurons in juvenile mice: implications for the timing of puberty. American Journal of Physiology-Endocrinology and Metabolism. 2009; 297(5): E1212-E1221. doi: 10.1152/ajpendo.00461.2009