Study on the mechanism of sarsasapogenin in treating precocious puberty by regulating HPG axis

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Research

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Abstract

Background Precocious puberty is a common endocrine disorder in children,and its pathogenic factor is early initiation of hypothalamic-pituitary-gonadal (HPG) axis. Sarsasapogenin is the main component of traditional Chinese medicine Zhimu, which has anti-fertility effect. There is already evidence that anti-fertility drugs may be resistant to precocity by regulating the HPG axis. Therefore, we speculated that sarsasapogenin might also has the effect. In order to test this hypothesis, this study determined the effect and mechanism of sarsasapogenin on precocious puberty by establishing a danazol-induced precocious puberty model.

Methods Female Sprague-Dawley rats were divided into normal(N)group, model (M) group, leuprolide (L) group and sarsasapogenin (Sar) group. Rats at 5 days of age were given a single subcutaneous injection of 300 microgram of danazol dissolved in 25 microliter vehicle of ethylene glycol-ethanol (1:1, v/v), to establish the precocious puberty model. After 10 days of modeling, drug intervention was started. Vaginal opening was started at the age of 20 days, and then vaginal cell smears were examined. The development of uterus and ovary was observed by hematoxylin and eosin (HE) staining. The levels of Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol (E2) were determined by radioimmunoassay. The expressions of hypothalamic gonadotropin releasing hormone (GnRH), Kiss-1, G protein-coupled receptor 54 (GPR54) and pituitary gonadotropin releasing hormone receptor (GnRH-R) were detected by RT-PCR.

Results The day of vaginal opening was significantly advanced in the M than that in the N group. Compared with the M group, the Sar and L groups could significantly delay the opening time of vaginal ($P < 0.001$,$P < 0.05$,respectively). The Sar group could significantly decrease uterine and ovarian coefficients, and reduce uterine wall thickness ($P < 0.05$, respectively). In terms of serum hormones (LH, FSH, E2), the M group was significantly higher than the N group ($P < 0.001$,respectively). Moreover, the Sar group can significantly down-regulate the levels of serum hormones ($P < 0.001$, $P < 0.01$, $P < 0.01$,respectively). Also, the expression of GnRH, GnRH-R, Kiss-1 mRNA were significantly decreased in the Sar group compared with that in the M group ($P < 0.01$, $P < 0.05$, $P < 0.001$, respectively).

Conclusions The results showed that sarsasapogenin had the effect of treating precocious puberty, and its mechanism might be to down-regulate the expression of GnRH and GnRH-R mRNA through the Kiss-/GPR54 system, thus delaying the initiation of HPG axis.

Background

Precocious puberty, a growth and development disorder, is caused by the premature activation of GnRH neurons[1]. It is defined as the appearance of secondary sex characteristics before 8 years of age in girls and before 9 years of age in boys[2]. A Danish study showed that the prevalence of precocious puberty in girls was 0.2%, that of boys was less than 0.05%, and the prevalence of girls was much higher than boys[3]. Children with precocious puberty tend to be short in stature due to early onset of puberty, early

growth and development, and shortened bone growth years[4]. In addition, precocious puberty can cause a series of psychological and physical problems in patients, and may be also associated with metabolic diseases such as diabetes, cardiovascular disease, breast cancer and prostate cancer[5, 6]. In recent years, studies have found that the age of onset of precocious puberty has been decreasing[7, 8], and it has become a social focus.

At present, gonadotropin releasing hormone analogue (GnRHa) are widely used to treat precocious puberty[9]. Leuprolide is the most commonly used. It acts on the pituitary in a continuous non-pulsed form, down-regulates its GnRH receptor or reduces its sensitivity to GnRH receptor, and inhibits the pituitary to secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH), thus inhibiting the development of secondary sex characteristics[10, 11], achieving the effect of therapeutic precocious puberty. Although leuprolide plays a significant role in the treatment of precocious puberty, it has been found that it is easy to cause vaginal bleeding when used for the first time, with an incidence of 16%-60% [12]. And it can also increase the incidence of polycystic ovary syndrome[13]. In recent years, traditional Chinese medicine has been successfully used to treat precocious puberty[14], which can achieve the purpose of delaying bone age maturity and benefit the final height of patients[15]. However, the current research on the therapeutic precocious puberty of traditional Chinese medicine is mainly focused on the compound, while for single chinses herb, especially the effective ingredients are few.

The traditional Chinese medicine Zhimu (Anemarrhena asphodeloides Bge.)is a dried rhizome of Liliaceae, which has the functions of clearing heat and purging fire, nourishing yin and moistening. Pharmacological studies show that Zhimu also has anti-thrombosis, ameliorating Alzheimer's disease, anti-tumor, anti-inflammatory, anti-depression and other effects[16, 17]. Through analyzing the relevant literature on the treatment of precocious puberty of Chinese medicine in the past 40 years, it is found that among the precocious puberty of Chinese medicine, Zhimu is used most frequently[18]. Recent studies have found that Zhimu can directly affect GnRH neurons and inhibit GnRH mRNA expression[19]. But the main components are unknown. Sarsasapogenin is a main active component isolated from Zhimu[20]. It has been proven that sarsasapogenin is similar in structure to the hormone backbone and can affect reproductive function in rats. Besides it has anti-fertility effects[21, 22]. It has been reported that anti-fertility drugs may be associated with the treatment of precocious puberty[23, 24]. For example, the traditional Chinese medicine Zicao (Radix Lithospermi), on the basis of its anti-fertility effect, was found to reduce LH, FSH and E_2 levels in rat serum by inhibiting the effect of hypothalamic-pituitary-gonadal (HPG) axis, thus delaying the time of vaginal opening and playing a role in treating sexual precocity. Therefore, we speculate that sarsasapogenin may be one of the ingredients in the resistance of precocious puberty. To test this hypothesis, we established a danazol-induced precocious puberty model to determine the effect and mechanism of sarsasapogenin.

Methods

Animals and experimental protocols

Twenty-four SPF grade Female Sprague-Dawley rats at 3 days of age in company with the maters were purchased from Beijing SiPeiFu biotechnology co., LTD (license: SYXK(JING)2016-0038). All animals were raised in room with 24 °C constant temperature (Humidity 42%), a 12-hour light/dark cycle, with food and water available ad libitum. All experimental procedures involving the animals were approved by the Experimental Animal Ethics Committee of the Academic Committee of Beijing University of Chinese Medicine. The rats were randomly divided into normal(N)group, model (M) group, leuprolid （L）group and sarsasapogenin (Sar) group. At the 5th day of age, except the N group, the other three groups were given a single subcutaneous injection of 300 μg of Danazol (Yuanye Bio-Technology Co., Ltd, Shanghai,China) dissolved in 25 μl vehicle of ethylene glycol-ethanol(1:1, v/v)[25]. After 10 days of modeling, the L group was subcutaneously injected with leuprolide (Livzon Pharmaceutical Co., Ltd., Shanghai, China) at a dose of 100ug/kg, and the Sar group was gavage with a dose of 4 mg/kg (Yuanye Bio-Technology Co., Ltd, Shanghai,China). The N and M groups were given the same amount of saline. At the age of 20 days, rats were inspected daily for vaginal opening, thereafter, vaginal smears were examined daily. When the first diestrus period appeared, rats were sacrificed and the other groups were randomly sacrificed at 1:1. All animals were weaned on day 23.

Estrous cycle determination

Animals with vaginal openings were given daily vaginal cell smears at 8:30 am, to makesure whether the rats were entering a regular estrous cycle. Vaginal cells were collected via saline lavage and then stained with 4% Methylene-blue (Yuanye Bio-Technology Co., Ltd, Shanghai,China). Generally, a regular estrous cycle includes proestrus, estrus, metestrus and diestrus. Nucleated epithelial cells were predominant in the proestrus stage; cornified squamous epithelial cells were predominant in the estrus stage; both cornified squamous epithelial cells and leukocytes indicated the metaestrus stage; and predominant leukocytes indicated the diestrus stage.

Hormonal analysis of serum

Rats were weighed and anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL / 100 g) before being sacrificed. Blood samples were taken from the abdominal aorta, serum was centrifugally collected at 4 °C, and stored at -20 °C until analysis. Serum hormones LH, FSH, and estradiol (E_2) levels were determined using radioimmunoassay kits (sinouk institute of biological technology Beijing,China) according to the manufacturer's specifications. The sensitivity of E_2 kit was 5 pg/mL, and the intra- and inter-assay coefficients of variation were < 10% and < 15.2%. The sensitivity of the LH kit was 0.2-5.0 mIU/mL, and the intra- and inter-assay coefficients of variation were 2.0-2.4% and 4.2–7.5%. The sensitivity of the FSH kit was 0.25 mIU/mL, and the intra- and inter-assay coefficients of variation were 2.2–2.5% and 3.7–8.7%.

Pathological examination of uterus and ovary

After the blood samples were taken, uterus and ovary samples were then rapidly removed from the animals, weighed, and the uterine and ovarian coefficients were calculated (mg/100 g). Subsequently, uterus and ovary samples were fixed with 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) according to the standard histological procedures. To measure the thickness of the uterine wall, we took pictures of 40 x field of view of each section. Make sure each photo has the same background light. Image-pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) software was used to measure the thickness of the uterine wall (mm) by taking the 40x ruler at the lower right corner as the standard and selecting 5 points for each section. And the corpus luteum of each ovary section was counted under the microscope.

Real-time fluorescence quantitative polymerase chain reaction (PT-PCR) analysis

The hypothalamus and pituitary were dissected from the brain to detect the mRNA expression of GnRH, Kiss-1, G protein-coupled receptor 54 (GPR54) in the hypothalamus and GnRH-R in the pituitary. The total RNA was extracted according to the instructions of HiPure Total RNA Mini Kit (MAGEN), and the RNA concentration was measured using a UV spectrophotometer (UV-2000, Unico, Shanghai, China). According to the instructions, reverse transcription was performed on a T100 Thermal Cycler PCR machine (Bio-Rad, USA) using the Reveraid First Strand cDNA Synthesis kit and the SYBR PCR master mix. Amplification and quantification were performed on a Real-Time PCR machine (Bio-Rad, USA). The RT-RCR method was as follows: initial denaturation at 95 °C for 10 min, then denaturation at 95 °C for 10 s, annealing at 55 °C (annealing temperature of Kiss-1/GPR54 is 60 °C), and extension for 30 s with a total of 50 amplification cycles. The β-actin gene was used as the internal standard. Using the $2^{-\Delta\Delta Ct}$ method to calculate the relative expression levels, and the primers were synthesized by Beijing Bomad Gene Technology Co., Ltd. (Beijing, China). The primers sequence is shown in Table 1.

Statistical analysis

Data are presented as mean ± SEM, and with normal distribution and homogeneity of variance were analyzed using Student's t-test. Wilcoxon rank sum test were used for data with non-normal distribution. Statistical analysis was performed using SAS 8.2 (IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

Time of vaginal opening and vaginal cell smear

A vaginal opening did not appear in the N group until 30 days of age. The vaginal opening time in the M group was significantly advanced than that in N group, indicating that the model of danazol-induced precocity was successfully established. Compared with the M group, the effect of delaying vaginal opening was significant in the L and Sar groups ($P < 0.05$, $P < 0.001$, respectively). (Fig. 1). And a regular estrous cycle was shown in the Fig. 2.

Effects of sarsasapogenin on uterine and ovarian coefficients

The results showed that the coefficients of uteri and ovaries in the M group increased significantly than that in N group ($P < 0.01$, $P < 0.05$, respectively). Compared with the M group, the uteri and ovaries coefficients of the L and the Sar group were significantly reduced ($P < 0.01$, $P < 0.05$, respectively). It means that the L and Sar groups can inhibit the development of gonadal, played a therapeutic role (Fig. 3).

Effects of sarsasapogenin on uterine wall thickness and ovarian luteinization rate

The pathological sections of the uterus showed that the M group had a clearer tissue hierarchy than the N group, and the cell morphology was normal without significant pathological changes (Fig. 4). Also, the thickness of the uterine wall in the M group was significantly increased than that in N group ($P < 0.05$). And the uterine wall thickness in the Sar group was significantly reduced compared with the M group ($P < 0.05$). The effect of reducing uterine wall thickness in the L group was the most significant ($P < 0.01$); (Fig. 5). Under the microscope, the luteinizing number of ovary in each section was observed. There were only one luteinizing number in the N group, five in the M group, two in the L group, and two in the Sar group. The difference was not statistically significant, therefore, graph analysis is not done in this study

Effects of sarsasapogenin on serum hormones

Compared with the N group, serum hormones in the M group were significantly increased ($P < 0.001$, respectively), consistent with the symptoms of precocious puberty. Compared with the M group, the levels of LH, FSH and E_2 in Sar were significantly decreased ($P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively). And the levels of LH, FSH and E_2 were also significantly decreased in the L than that in the M group ($P < 0.01$, $P < 0.05$ and $P < 0.01$, respectively). And from the average value of the ordinate, the inhibitory effect of sarsasapogenin on serum hormone is better than that of leuprolide (Fig. 6).

Effects of sarsasapogenin on hypothalamic GnRH and pituitary GnRH-R mRNA expression.

Compared with the N group, the expression of GnRH and GnRH-R mRNA were significantly increased in the M group ($P < 0.001$, $P < 0.05$,respectively). Compared with the M group, the L group significantly reduced the expression of GnRH and GnRH-R ($P < 0.01$). And it has significant inhibitory effect on GnRH-R ($P < 0.001$), which is consistent with the mechanism of leuprolide. After treatment with sarsasapogenin, the mRNA expression of GnRH and GnRH-R decreased significantly ($P < 0.01$, $P < 0.05$, respectively). These results indicate that sarsasapogenin may act on the HPG axis to treat precocious puberty (Fig. 7).

Effects of sarsasapogenin on expression of Kiss-1 and GPR54 mRNA in hypothalamus

Compared with the N group, the mRNA expressions of Kiss-1 and GPR54 in the M group were significantly increased ($P < 0.001$). Compared with the M group, the levels of Kiss-1 mRNA in the L and Sar groups were significantly reduced ($P < 0.001$, $P < 0.001$, respectively). However, the expression of GPR54 mRNA showed that only L group was significantly reduced ($P < 0.001$), while there was no significant difference between Sar and M groups (Fig. 8).

Discussion

The incidence of precocity has been on the rise in recent years[26], which can be clinically subdivided into true (central) precocity and peripheral precocity, with true precocity being the most common[27]. Although the main causes of different genders are different, the development process of true precocious puberty is consistent with normal puberty[28–30]. Initiation of puberty occurs by activating the HPG axis. GnRH pulsed release promotes gonadotropin secretion. It then acts on the gonads and promotes the secretion of sex hormones, finally leading to the maturation of the sexual organs and entering puberty[31]. Precocious puberty is caused by the early initiation of puberty.

Puberty is a complex developmental process regulated by multiple genetic and neuroendocrine factors[32, 33]. The hypothalamus of rats is generally considered immature within 1–10 days after birth[34, 35], and the maturation of the HPG axis can be regulated by administration. Danazol, a derivative of 17α-acetylene testosterone, has weak androgenic activity and has effect on the reproductive system of rats[25]. Studies have shown that the administration of danazol to newborn rats can rapidly promote the activation of the HPG axis[36], making them enter puberty earlier. Therefore, in this study, female SD rats at 5 days of danazol administration were used as the model to analyze the true precocious puberty.

To determine whether the model is entering puberty, we measured vaginal openings, serum hormones, uterine and ovarian coefficients. The vaginal opening is a sign of the beginning of puberty. Our results showed that the vaginal opening of the M group was significantly advanced than that in N group, and uterine and ovarian coefficients were significantly increased, no obvious pathological changes were found in pathological sections. This indicates that the model of precocious puberty was successfully constructed. And the Sar group had a significant delaying effect compared with the M group, indicating that sarsasapogenin has a therapeutic effect on precocious puberty.

LH and FSH are glycoprotein hormones synthesized by gonadotropins in the anterior pituitary gland, which have significant effects on adolescent development, gonadal and reproductive functions. Premature secretion of LH and FSH can lead to early gonadal activation[37, 38]. Estrogen is a steroid hormone secreted by the ovary[39], and its receptors are distributed in the uterus, breast and other parts, which can promote the maturation of the sexual organs[40]. In addition, estradiol is also an important

hormone regulating the growth of height during puberty, which can stimulate growth by stimulating the secretion of growth hormone (GH)[41]. Premature estrogen secretion can accelerate bone maturation, shorten growth cycle and reduce the final height of adults[42]. Serum hormone is the most intuitive indicator of clinical efficacy[43–45]. Our results showed that sarsasapogenin could significantly reduce serum hormone levels, and from the numerical point of view, the inhibitory effect of sarsasapogenin on serum hormone is better than that of leuprolide.

GnRH is a small decapeptide that serves as an important connection between the neural and endocrine systems[46]. It acts on anterior pituitary gonadotropes, which express GnRH-R. Driving pituitary gonadotropic hormone release (GTH), including LH and FSH, then gonadotropin acts on the ovaries or testes to secrete sex hormones such as estradiol or aldosterone, which promotes gonad development and eventually enters puberty[47–50]. We detect the mRNA expression of GnRH and GnRH-R by RT-PCR. The results showed that sarsasapogenin could significantly reduce both of the expression, indicating that sarsasapogenin could inhibit related hormones by inhibiting the release of GnRH. However, in terms of GnRH-R inhibition, the effect of leuprolide was more significant. This is consistent with its mechanism, which has the function of inhibiting pituitary and gonadal development. In addition, continuous administration of leuprolide to replace the GnRH pulsed release will result in the down-regulation of GnRH-R, which will further inhibit the release of gonadotropin[10, 51].

GnRH is the enabler and core substance of HPG axis[52], and the activation of this neuron is regulated by a variety of neuropeptides. Many studies have shown that the Kiss-1/GPR54 system is closely related to the release of GnRH. The Kiss-1 was first discovered in malignant melanoma cells in 1996[53], and the expression of Kiss-1 mRNA was significantly increased in pubery rats[54, 55]. By injecting kisspeptin-10 into sheep's brain, a large amount of GnRH can be directly observed in the cerebrospinal fluid, accompanied by an increase in LH[56]. This effect is caused by the Kiss-1 encoded product, kisspeptin, which binding to the GPR54[57]. GPR54 is a G protein-coupled receptor in the rhodopsin family[58]. People with GPR54 dysfunction cause hypogonadism. By using double-label in situ hybridization, it was found that 77% of the GnRH neurons coexpress GPR54 mRNA[59]. It means that the Kiss-1/GPR54 system may directly act on GnRH neurons to promote the secretion of GnRH.

Therefore, the mRNA expression of Kiss-1 and GPR54 were detected. The results showed that Kiss-1 mRNA in L and Sar groups were significantly reduced than those in the M group. And, unlike leuprolide, GPR54 mRNA levels in the Sar group did not differ significantly compared to the M group. It has been reported that the effect of GPR54 on puberty development is not only related to the expression of GPR54 mRNA, but also related to the functional characteristics of GPR54. Therefore, as long as the GPR54 receptor function is normal and has the property of high binding with the ligand, it can still cause the excitation of GnRH neurons[60]. So, it is not ruled out that sarsasapogenin may exert resistance and precocity through the Kiss-1/ GPR54 system.

In summary, sarsasapogenin can down-regulate the expression of GnRH and GnRH-R through the Kiss-1/GPR54 system, reduce serum hormone levels, and inhibit the development of gonads, thereby delaying the activation of the HPG axis and exerting the effect of therapeutic precocity. Also, sarsasapogenin is a major active ingredient in Zhimu, which can be taken orally [61, 62], is safe, has a similar effect to leuprorelin. So it has good development prospect

Abbreviations

HPG
Hypothalamic-pituitary-gonadal axis
GnRHa
Gonadotropin releasing hormone analogue.
LH
Luteinizing Hormone
FSH
Follicle-Stimulating Hormone
E2
Estradiol
GnRH
Gonadotropin-releasing hormone
GNRH-R
Gonadotropin-releasing hormone receptor
GPR54
G protein-coupled receptor 54
GH
Growth hormone
GTH
Gonadotropic hormone

Declarations

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Authors'contributions

KLH and WYS made contributions to conception, design and analysis of data; participated in drafting of the manuscript. YL made contributions to the model building and manuscript modification. BZ, MZ, and CYG investigated and read relevant background documents; participated in the revision of the manuscript. And HSC made contributions to the design of the experiment; Been involved in the revision of

important intellectual content of the manuscript. XLW made contributions to revise the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics declarations

Ethics approval and consent to participate

The study was approved by the Experimental Animal Ethics Committee of the Academic Committee of Beijing University of Chinese Medicine.

Consent for publication

Yes

Competing interests

The authors declare that they have no competing interests.

References

1. Bridges NA, Christopher JA, Hindmarsh PC, Brook CG. Sexual precocity: sex incidence and aetiology. Arch Dis Child. 1994;70:116-8.
2. Soriano-Guillén L, Argente J. Central precocious puberty, functional and tumor-related. Best Pract Res Cl En. 2019;33:101262.
3. Sørensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A. Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. Horm Res Paediat. 2012;77:137-45.
4. Guaraldi F, Beccuti G, Gori D, Ghizzoni L. MANAGEMENT OF ENDOCRINE DISEASE: Long-term outcomes of the treatment of central precocious puberty. Eur J Endocrinol. 2016;174:R79-R87.
5. Rostami S, Kohan L, Mohammadianpanah M. The LEP G-2548A gene polymorphism is associated with age at menarche and breast cancer susceptibility. Gene. 2015;557:154-7.
6. Day FR, Thompson DJ, Helgason H, Chasman DI, Finucane H, Sulem P, Ruth KS, Whalen S, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. Nat Genet. 2017;49:834-41.
7. McDowell MA, Brody DJ, Hughes JP. Has Age at Menarche Changed? Results from the National Health and Nutrition Examination Survey (NHANES) 1999–2004. J Adolesc Health. 2007;40:227-31.
8. Soriano-Guillén L, Corripio R, Labarta JI, Cañete Rn, Castro-Feijóo L, Espino R, Argente Js. Central Precocious Puberty in Children Living in Spain: Incidence, Prevalence, and Influence of Adoption and Immigration. J Clin Endocrinol Metab. 2010;95:4305-13.
9. Chen M, Eugster EA. Central Precocious Puberty: Update on Diagnosis and Treatment. Paediatr Drugs. 2015;17:273-81.
10. Kendirci HN, Agladioglu SY, Onder A, Bas VN, Cetinkaya S, Aycan Z. Effects of GnRH analogue treatment on anterior pituitary hormones in children with central precocious puberty. J Pediatr Endocrinol Metab. 2015;28:1145-51.
11. Wallach EE, Yen SSC. Clinical applications of gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs. Fertil Steril. 1983;39:257-66.
12. Li WJ, Gong CX, Guo MJ, Xing J, Li T, Song WH, Luo XP, Wu D, et al. Efficacy and Safety of Domestic Leuprorelin in Girls with Idiopathic Central Precocious Puberty: A Multicenter, Randomized, Parallel, Controlled Trial. Chin Med J (Engl). 2015;128:1314-20.
13. Cantas-Orsdemir S, Eugster EA. Update on central precocious puberty: from etiologies to outcomes. Expert Rev Endocrinol Metab. 2019;14:123-30.
14. Lin Y-C, Chang T-T, Chen H-J, Wang C-H, Sun M-F, Yen H-R. Characteristics of traditional Chinese medicine usage in children with precocious puberty: A nationwide population-based study. J Ethnopharmacol. 2017;205:231-9.
15. Yu C-H, Liu P-H, Van Y-H, Lien AS-Y, Huang T-P, Yen H-R. Traditional Chinese medicine for idiopathic precocious puberty: A hospital-based retrospective observational study. Complement Ther Med.

2014;22:258-65.

16. Wang Y, Dan Y, Yang D, Hu Y, Zhang L, Zhang C, Zhu H, Cui Z, et al. The genus Anemarrhena Bunge: A review on ethnopharmacology, phytochemistry and pharmacology. J Ethnopharmacol. 2014;153:42-60.
17. Zhao X, Liu C, Qi Y, Fang L, Luo J, Bi K, Jia Y. Timosaponin B-II ameliorates scopolamine-induced cognition deficits by attenuating acetylcholinesterase activity and brain oxidative damage in mice. Metab Brain Dis. 2016;31:1455-61.
18. Llu Z, Liu L, Jing W, Qu W, Wang Y. Analysis on medication rule of Chinese medicine in treating precocious puberty based on data mining technology. Chin Med Herald (in Chinese). 2019;16:120-3+36.
19. Lee SH, Kwak SC, Kim DK, Park SW, Kim HS, Kim YS, Lee D, Lee JW, et al. Effects of Huang Bai (Phellodendri Cortex) and Three Other Herbs on GnRH and GH Levels in GT1–7 and GH3 Cells. Evid Based Complement Alternat Med. 2016;2016:9389028.
20. Bao W, Pan H, Lu M, Ni Y, Zhang R, Gong X. The apoptotic effect of sarsasapogenin from Anemarrhena asphodeloides on HepG2 human hepatoma cells. Cell Biol Int. 2007;31:887-92.
21. El-Ishaq A, Alshawsh MA, Chik ZB. Evaluating the oestrogenic activities of aqueous root extract of Asparagus africanus Lam in female Sprague-Dawley rats and its phytochemical screening using Gas Chromatography-Mass Spectrometry (GC/MS). PeerJ. 2019;7:7254.
22. Tafesse G, Mekonnen Y, Makonnen E. Antifertility effect of aqueous and ethanol extracts of the leaves and roots of Asparagus africanus in rats. Afr Health Sci. 2006;6:81-5.
23. Yan. Z. Experimental study on the inhibition on the hpg axis of pubertal rats by arnebia. master. Chongqing Medical University (in Chinese); 2007.
24. Song L. The effect of lithospermum extracts treatment on experimental true precocious puberty. master. Chongqing Medical University (in Chinese); 2006.
25. Morishita H, Takemoto M, Kondo H, Higuchi K, Aono T. Induction of true precocious puberty by neonatal treatment with danazol in female rats. Neurosci Lett. 1993;157:33-6.
26. Sømod ME, Vestergaard ET, Kristensen K, Birkebæk NH. Increasing incidence of premature thelarche in the Central Region of Denmark - Challenges in differentiating girls less than 7 years of age with premature thelarche from girls with precocious puberty in real-life practice. Int J Pediatr Endocrinol. 2016;2016:4.
27. Chirico V, Lacquaniti A, Salpietro V, Buemi M, Salpietro C, Arrigo T. Central precocious puberty: from physiopathological mechanisms to treatment. J Biol Regul Homeost Agents. 2014;28:367-75.
28. Mogensen SS, Aksglaede L, Mouritsen A, Sørensen K, Main KM, Gideon P, Juul A. Diagnostic work-up of 449 consecutive girls who were referred to be evaluated for precocious puberty. J Clin Endocrinol Metab. 2011;96:1393-401.
29. Aguirre RS, Eugster EA. Central precocious puberty: From genetics to treatment. Best Pract Res Cl En. 2018;32:343-54.

30. Jakubowska A, Grajewska-Ferens M, Brzewski M, Sopyło B. Ussefulnes of imaging techniques in the diagnostics of precocious puberty in boys. Pol J Radiol. 2011;76:21-7.
31. Osman HA, Al-Jurayyan NAM, Babiker AMI, Al-Otaibi HMN, AlKhalifah RDH, Al Issa SDA, Mohamed S. Precocious puberty: An experience from a major teaching hospital in Central Saudi Arabia. Sudan J Paediatr. 2017;17:19-24.
32. Brown DB, Loomba-Albrecht LA, Bremer AA. Sexual precocity and its treatment. World J Pediat. 2013;9:103-11.
33. Leka-Emiri S, Chrousos GP, Kanaka-Gantenbein C. The mystery of puberty initiation: genetics and epigenetics of idiopathic central precocious puberty (ICPP). J Endocrinol Invest. 2017;40:789-802.
34. Daikoku S, Morishita H, Hashimoto T, Takahashi A. Light-microscopic studies on the development of the interrelationship between the neurosecretory pathway and the portal system in rats. Endocrinol Jpn. 1967;14:209-24.
35. Dussault JH, Walker P, Dubois JD, Labrie F. The development of the hypothalamo–pituitary axis in the neonatal rat: sexual maturation in male and female rats as assessed by hypothalamic LHRH and pituitary and serum LH and FSH concentrations. Can J Physiol Pharmacol. 1977;55:1091-7.
36. Tian Z, Zhao H, Sun Y, Cai D, Chen B. Evaluation of the true precocious puberty rats induced by neonatal administration of Danazol: Therapeutic effects of nourishing "Yin"- removing "Fire" Chinese herb mixture. Reprod Biol Endocrinol. 2005;3:38.
37. Coss D. Regulation of Reproduction via Tight Control of Gonadotropin Hormone Levels. Mol Cell Endocrinol. 2018;463:116-30.
38. Huhtaniemi I. Mutations along the pituitary–gonadal axis affecting sexual maturation: Novel information from transgenic and knockout mice. Mol Cell Endocrinol. 2006;254-255:84-90.
39. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends Mol Med. 2013;19:197-209.
40. Hewitt SC, Korach KS. Estrogen Receptors: New Directions in the New Millennium. Endocr Rev. 2018;39:664-75.
41. Juul A. The effects of oestrogens on linear bone growth. APMIS. 2001;109:124-34.
42. Tencer J, Lemaire P, Brailly-Tabard S, Brauner R. Serum inhibin B concentration as a predictor of age at first menstruation in girls with idiopathic central precocious puberty. PLoS One. 2018;13:e0205810.
43. Lee HL, Lee YB, Choi JY, Lee JA. Herbal medicine for idiopathic central precocious puberty: A protocol for a systematic review of controlled trials. Medicine (Baltimore). 2018;97.
44. Silverman LA, Neely EK, Kletter GB, Lewis K, Chitra S, Terleckyj O, Eugster EA. Long-Term Continuous Suppression With Once-Yearly Histrelin Subcutaneous Implants for the Treatment of Central Precocious Puberty: A Final Report of a Phase 3 Multicenter Trial. J Clin Endocrinol Metab. 2015;100:2354-63.

45. Heo S, Lee YS, Yu J. Basal serum luteinizing hormone value as the screening biomarker in female central precocious puberty. Ann Pediatr Endocrinol Metab. 2019;24:164-71.
46. Plant TM. Neuroendocrine control of the onset of puberty. Front Neuroendocrinol. 2015;38:73-88.
47. Kletter GB, Klein KO, Wong YY. A Pediatrician's Guide to Central Precocious Puberty. Clin Pediatr. 2015;54:414-24.
48. Urbanski HF, Ojeda SR. Activation of Luteinizing Hormone-Releasing Hormone Release Advances the Onset of Female Puberty. Neuroendocrinology. 1987;46:273-6.
49. Dutlow CM, Rachman J, Jacobs TW, Millar RP. Prepubertal increases in gonadotropin-releasing hormone mRNA, gonadotropin-releasing hormone precursor, and subsequent maturation of precursor processing in male rats. J Clin Invest. 1992;90:2496-501.
50. Wilson AC, Meethal SV, Bowen RL, Atwood CS. Leuprolide acetate: a drug of diverse clinical applications. Expert Opin Investig Drugs. 2007;16:1851-63.
51. Li P, Li Y, Yang CL. Gonadotropin Releasing Hormone Agonist Treatment to Increase Final Stature in Children With Precocious Puberty: A Meta-Analysis. Medicine (Baltimore). 2014;93.
52. Stamatiades GA, Kaiser UB. Gonadotropin regulation by pulsatile GnRH: Signaling and gene expression. Mol Cell Endocrinol. 2018;463:131-41.
53. Lee J-H, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR. KiSS-1, a Novel Human Malignant Melanoma Metastasis-Suppressor Gene. J Natl Cancer I. 1996;88:1731-7.
54. Kuohung W, Kaiser UB. GPR54 and KiSS-1: Role in the regulation of puberty and reproduction. Rev Endocr Metab Disord. 2006;7:257-63.
55. Yang R, Wang Y-M, Zhang L, Zhao Z-M, Zhao J, Peng S-Q. Prepubertal exposure to an oestrogenic mycotoxin zearalenone induces central precocious puberty in immature female rats through the mechanism of premature activation of hypothalamic kisspeptin-GPR54 signaling. Mol Cell Endocrinol. 2016;437:62-74.
56. Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci U S A. 2005;102:1761-6.
57. Trevisan CM, Montagna E, de Oliveira R, Christofolini DM, Barbosa CP, Crandall KA, Bianco B. Kisspeptin/GPR54 System: What Do We Know About Its Role in Human Reproduction? Cell Physiol Biochem. 2018;49:1259-76.
58. Lee DK, Nguyen T, O'Neill GP, Cheng R, Liu Y, Howard AD, Coulombe N, Tan CP, et al. Discovery of a receptor related to the galanin receptors. FEBS Lett. 1999;446:103-7.
59. Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK, Steiner RA. Kisspeptin Activation of Gonadotropin Releasing Hormone Neurons and Regulation of KiSS-1 mRNA in the Male Rat. Neuroendocrinology. 2004;80:264-72.
60. Wacker JL, Feller DB, Tang XB, DeFino MC, Namkung Y, Lyssand JS, Mhyre AJ, Tan X, et al. Disease-causing Mutation in GPR54 Reveals the Importance of the Second Intracellular Loop for Class A G-

protein-coupled Receptor Function. J Biol Chem. 2008;283:31068-78.
61. Yang B, Liu Z, Hu J, Lai X, Xia P. Quantitative determination of sarsasapogenin in rat plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B. 2016;1022:213-9.
62. Wang J, Liu J, Guo X, Zhang J, Yu B. A sensitive indirect competitive enzyme-linked immunosorbent assay for the detection of sarsasapogenin in rat plasma. Acta Pharmacol Sin. 2010;31:984-9.

Table 1

Due to technical limitations, table 1 is only available as a download in the supplemental files section.

Figures

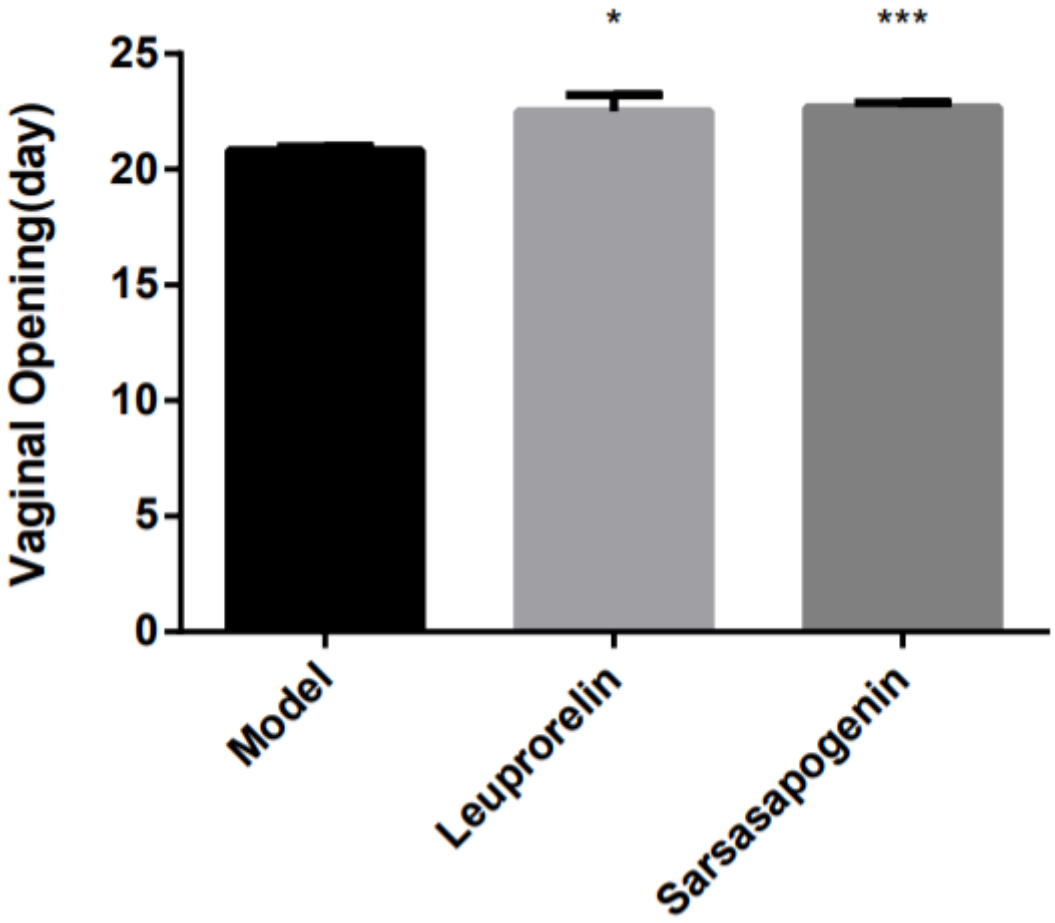

Figure 1

Time of vaginal opening. Data are expressed as mean ± SEM (n = 6/group). *P < 0.05, ***P < 0.001 vs. the M group.

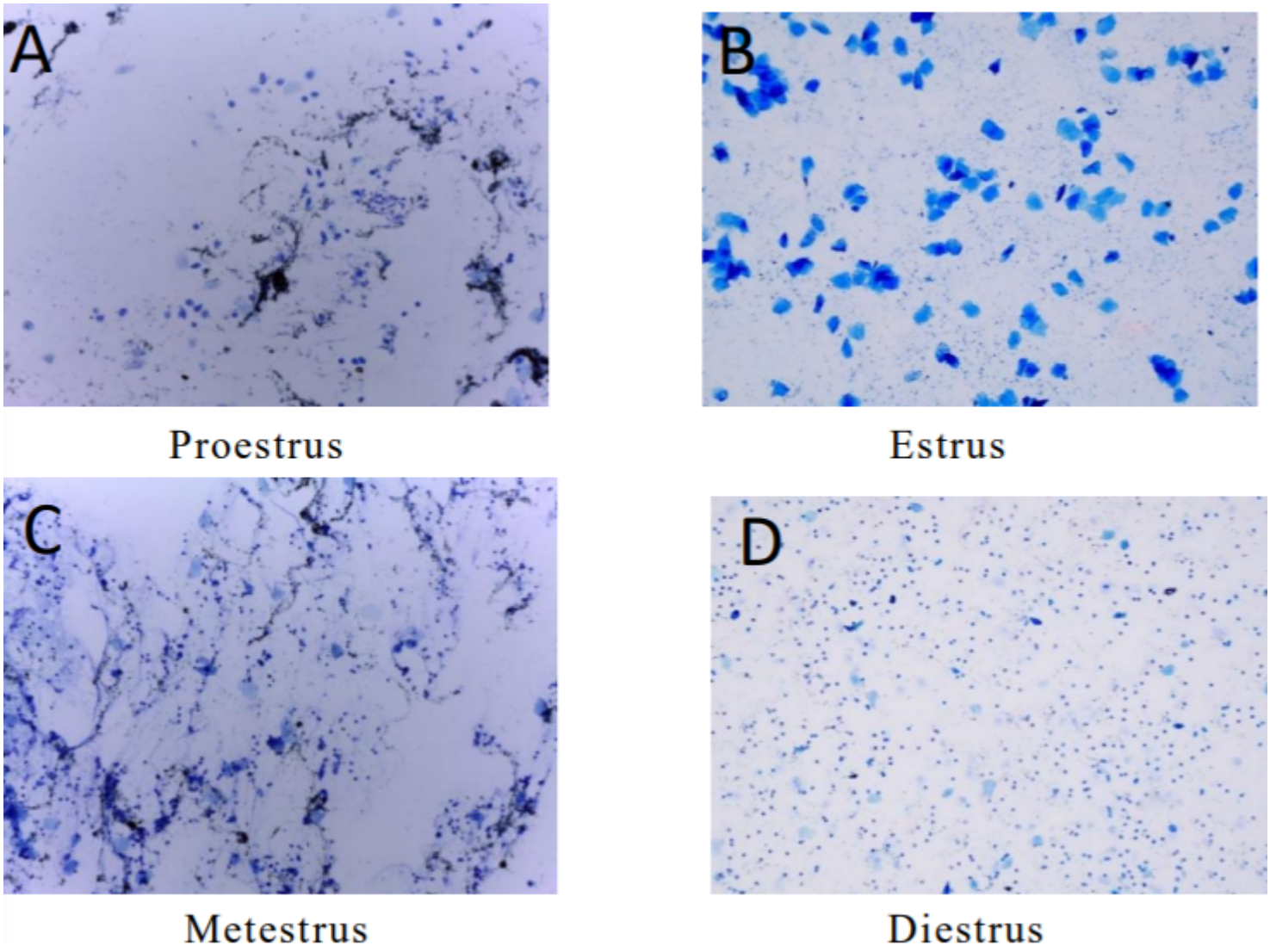

Figure 3

A regular estrous cycle. (20 x field of view)

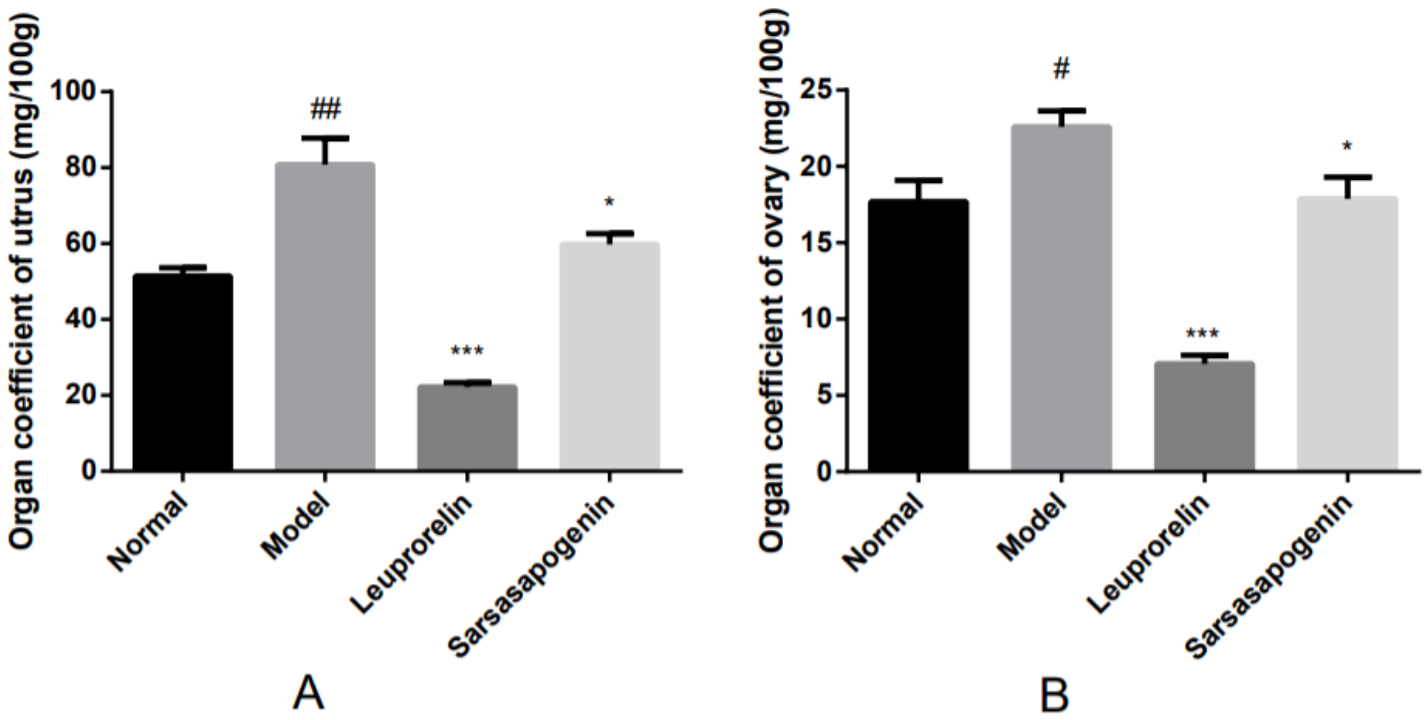

Figure 5

Uterine and ovarian coefficients. Data are expressed as mean ± SEM (n = 6/group). *P < 0.05 ***P < 0.001 vs. the M group; #P < 0.05 ##P < 0.01 vs. the N group.

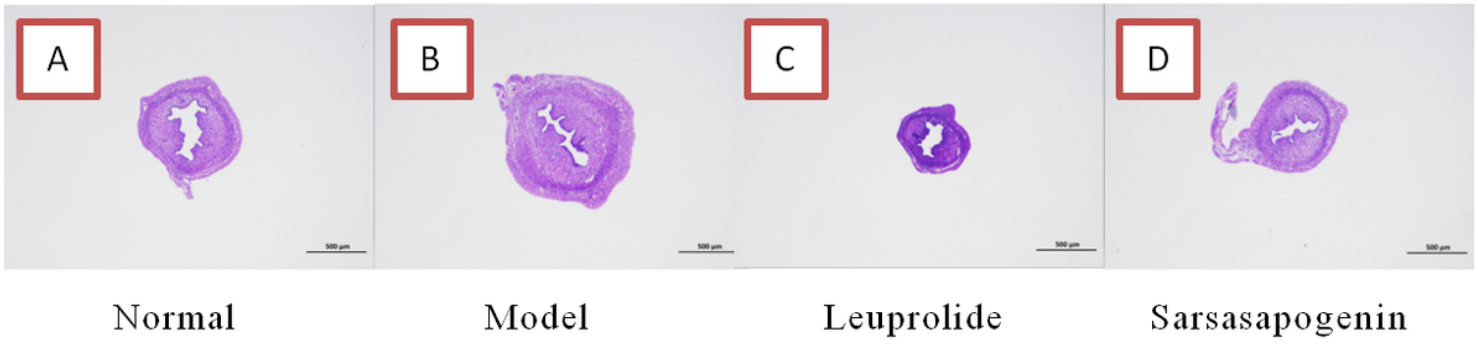

Figure 7

Uterine pathology section.

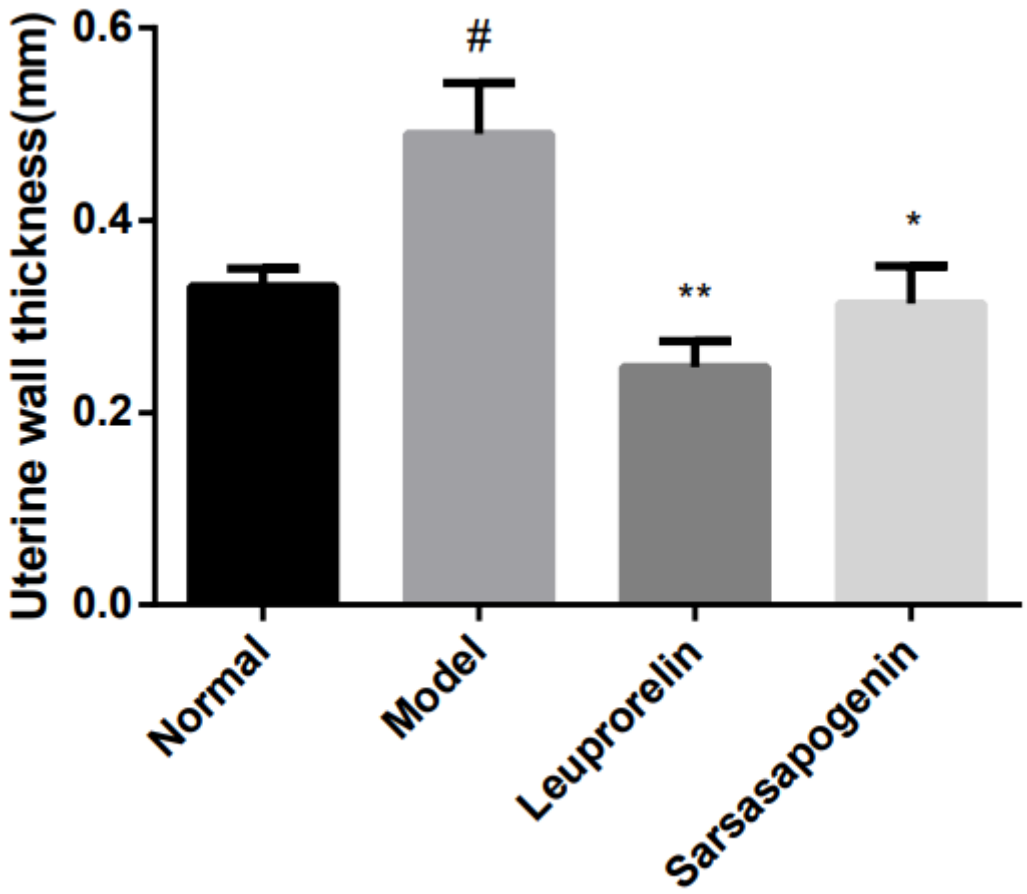

Figure 9

Uterine wall thickness. Data are expressed as mean ± SEM (n = 6/group). *P < 0.05 **P < 0.01 vs. the M group; #P < 0.05 vs. the N group.

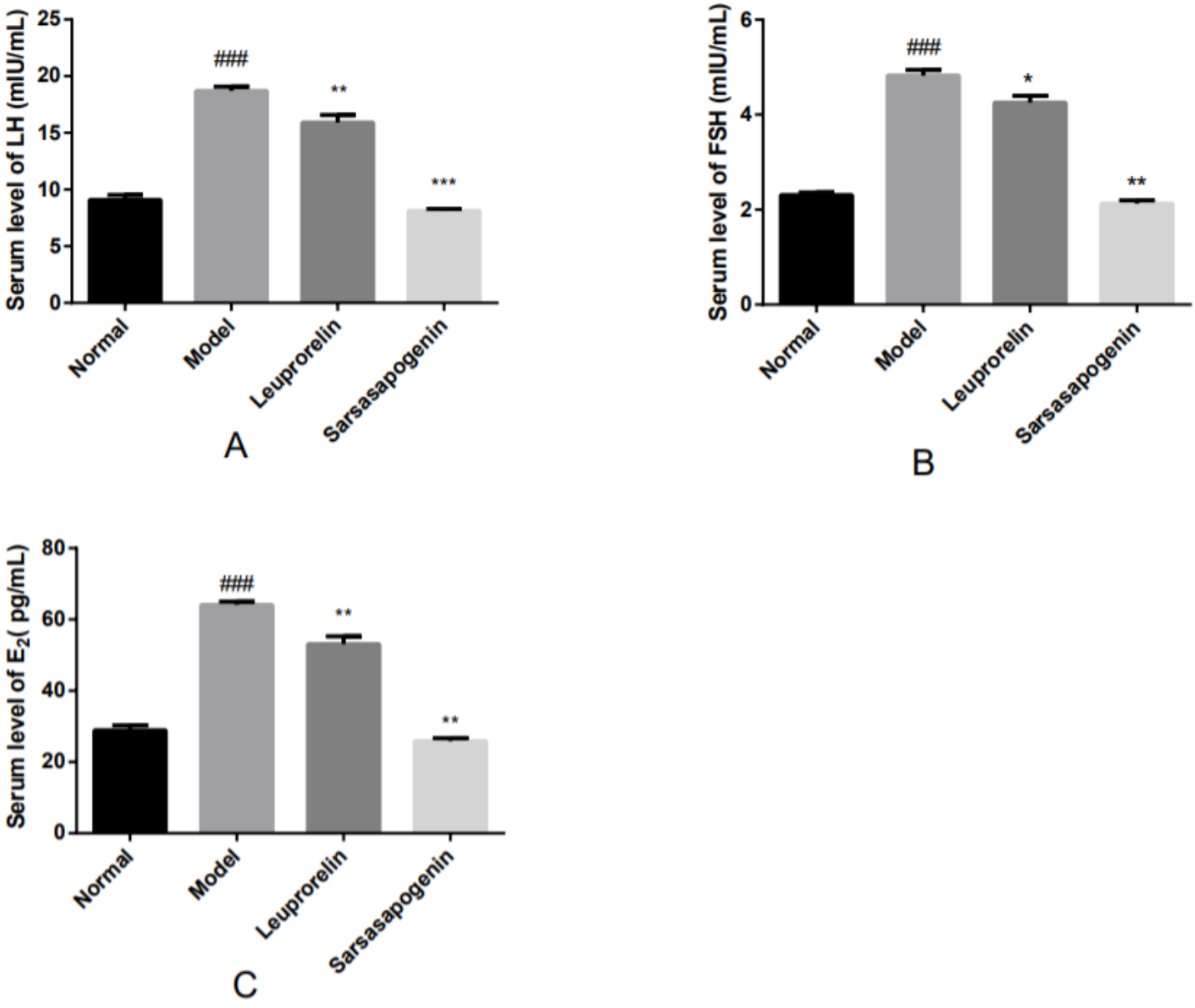

Figure 11

Serum hormone content. Data are expressed as mean ± SEM (n = 6/group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. the M group; ###P < 0.001 vs. the N group.

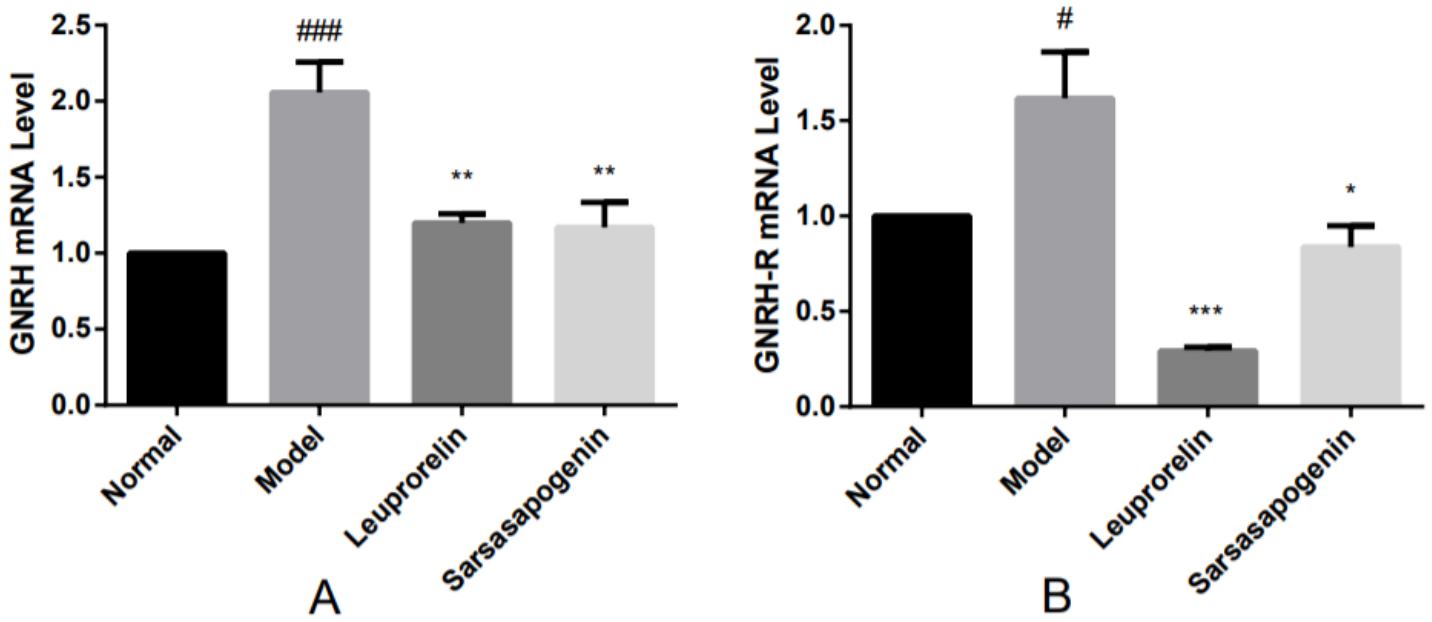

Figure 13

The expression levels of GnRH and GnRH-R mRNA. Data are expressed as mean ± SEM (n = 6/group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. the M group; #P < 0.05, ###P < 0.001 vs. the N group.

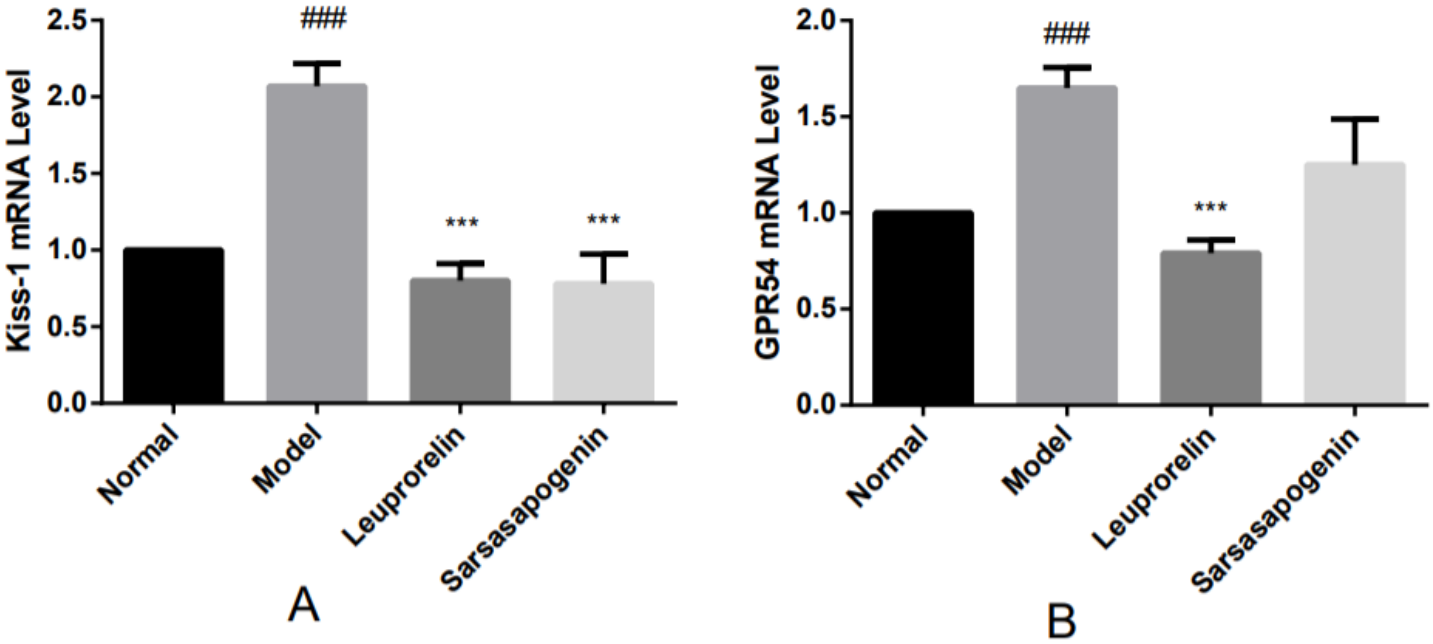

Figure 15

The mRNA expression of Kiss-1and GPR54 in hypothalamus. Data are expressed as mean ± SEM (n = 6/group). ***P < 0.001 vs. the M group; ###P < 0.001 vs. the N group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.PNG
- Table1.PNG