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Research article

Regulation of 11β-HSD1 reductase and the HPA axis by long-snake moxibustion in kidney-yang deficiency rats

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ABSTRACT

Background: Long-snake moxibustion can improve hypothalamic-pituitary-adrenal (HPA) axis function in patients with kidney-yang deficiency (KYDS). 11β-HSD1 controls the HPA axis by boosting CORT production via reductase activity. However, the interaction and mechanism of long snake moxibustion and 11β-HSD1 remain unknown. This study examined the impact of lengthy snake moxibustion on the hypothalamus-pituitary-adrenal axis in KYDS rats. The potential significance of 11β-HSD1 in this process was explored.

Methods: Rats were randomly divided into two groups: the blank group and the experimental group. The KYDS model was established with an intramuscular injection of hydrocortisone. Rats were randomly assigned to four groups: model, sham intervention, long snake moxibustion, and long snake moxibustion plus 11β-HSD1 inhibitor. Physical indicators included body weight, toe temperature, rectal temperature, and spontaneous movement. The serum levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and CORT were measured. Immunohistochemical examination reveals 11β-HSD1 protein expression in the liver. Western blotting (WB) detected the levels of 11β-HSD1, H6PDH and NADPH/NADP + protein in the liver.

Results: The experimental rats' body weight, toe temperature, rectal temperature, time and frequency of spontaneous activity all dropped, as did their serum ACTH, CORT, and CRH levels. The protein expressions of 11β-HSD1, H6PDH, and NADPH/NADP+ in the liver decreased significantly. Long-snake moxibustion improved HPA axis function in rats, boosting expression of 11β-HSD1, H6PDH, and NADPH/NADP+. Adding an 11β-HSD1 inhibitor to Long-snake moxibustion decreased its effect on the HPA axis.

Conclusion: Long-snake moxibustion improves KYDS symptoms in rats by increasing 11β-HSD1 expression and reductase activity, which regulates the HPA axis.

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1. Introduction

Yang deficiency constitution is one of the nine constitutions in Traditional Chinese Medicine (TCM). Epidemiological studies have found that individuals with Yang deficiency are prone to various diseases and experience accelerated disease progression, making it a significant factor in the occurrence, development, and recovery of these diseases [1–4]. Both populations that have transitioned from a mild constitution to a biased constitution, and those who persistently have a biased constitution have higher incidences of new diseases compared to other populations. This suggests that a biased constitution may be a precursor to disease occurrence and progression [5]. Kidney Yang, also known as Yuan Yang, true Yang, or true fire, is the foundation of all yang energies in the human body. By analyzing the pulse diagnosis pulse diagnosis characteristics of individuals with Yang deficiency, it has been found that the essence of these individuals is Kidney Yang deficiency, or Kidney Yang deficiency syndrome (KYDS) [6]. Currently, moxibustion treatments that warm and supplement Yang energy and unblock meridians are the primary therapeutic methods for addressing Yang deficiency. Long-snake moxibustion is a unique Chinese medicine moxibustion technique known for its extensive treatment areas and strong warming effects on Yang, demonstrating significant therapeutic benefits for Yang deficiency diseases [7–10]. Long-snake moxibustion was found to effectively increase the serum levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and corticosterone (CORT) in individuals with Yang deficiency, regulate the hypothalamic-pituitary-adrenal (HPA) axis, and improve clinical symptoms [11]. This effect may represent an important mechanism of action for long-snake moxibustion in treating Yang deficiency.

The HPA axis is an essential neuroendocrine-immune regulatory system that maintains internal homeostasis and modulates the body's protective response to both internal and external stressors. When the HPA axis, which is comprises the HPA glands, is activated, endocrine-related neurons synthesize and secrete CRH, which in turn stimulates the synthesis and release of ACTH and CORT. Modern research shows that dysfunction in various aspects and degrees of the HPA axis leading to decreased CORT levels is one of the main pathological features of KYDS [12]. 11β-hydroxy steroid dehydrogenase type 1 (11β-HSD1) is a key enzyme for glucocorticoid metabolism with bidirectional activity. It primarily functions as a reductase in vivo, converting non-bioactive dehydrocorticosterone into biologically active CORT [13,14].11β-HSD1 has been extensively studied in metabolic diseases, it has been involved in the occurrence and development of obesity, type 2 diabetes, hypertension, and other diseases; and can be a therapeutic target for metabolic diseases [15]. Although some studies have shown that 11β-HSD1 can regulate the HPA axis and that its activity is an essential part of this axis [16], its role in Yang deficiency syndrome remains unclear. Whether its reductase activity is related to the regulation of the HPA axis in KYDS by long-snake moxibustion needs further research.

This study aimed to characterize the changes in the HPA axis and the expression of 11β-HSD1 in a rat model of KYDS following long-snake moxibustion treatment. It also sought to investigate whether the reductase activity of 11β-HSD1 is involved in the regulatory effects of long-snake moxibustion on the HPA axis in KYDS. To the best of our knowledge, this is the first study to explore the role of 11β-HSD1 reductase in the treatment of renal KYDS in rats using long-snake moxibustion.

2. Materials and methods

2.1. Experimental animals

The animals used in the experiments were specific pathogen-free (SPF)-grade male Sprague–Dawley rats purchased from SPF Biotechnology Co., Ltd. (Beijing) (license no. SCXK: 2019–0010). The Ethics Committee of Jiangxi Zhonghong Boyuan Biotechnology Co., Ltd. Ethics Committee examined and approved the study scheme (ethics approval number 2020083001). The rats were 6 weeks old, weighed 200 ± 20 g, and were housed at 20°C-26 °C with a humidity level of 40%–70 %.

2.2. Experimental reagents and instruments

The moxa were purchased from Bozhou Traditional Chinese Medicine Factory (Anhui, China). Hydrocortisone (batch number: 830U051) was purchased from Solarbio (Beijing, China). 11β-HSD1 (batch number: DF3972)was purchased from Affinit (Jiangsu, China). Horseradish peroxidase-labeled goat anti-rabbit IgG (H + L) (batch number: ZB-2301)was purchased from Zhongshan Golden Bridge Biotechnology (Beijing, China). DAB chromogenic kit (batch number: CW0125), neutral resin (batch number: CW0136), Trizon Reagent (batch number: CW0580S), ultrapure RNA/miRNA extraction kit (batch number: CW0581M/CW0627) were purchased from CWBIO(Wuhan, China). Hematoxylin staining (batch number:AR1180-1) was purchased from Boster (Wuhan, China). ECL enhanced chemiluminescent solution (batch number: RJ239676) was purchased from ThermoFisher (Shanghai, China). BCA protein quantitation kit (batch number:E-BC-K318-M) was purchased from Elabscience (Wuhan, China). Hexose-6-phosphate dehydrogenase (H6PDH; batch number:ab170895), NADPH/NADP+ (batch number:ab133303) were purchased from Abcam (Shanghai, China). Mouse Anti-β-Actin (batch number:HC201) was purchased from TransGen Biotech (Beijing, China). Microscope (model: BX43) was purchased from Olympus (Tokyo, Japan). Automatic Microplate Reader (model: SuPerMax 3100) was purchased from Shanpu (Shanghai, China). Protein vertical electrophoresis apparatus (model: DYY-6C) was purchased from LiuYi (Beijing, China). Ultra-high sensitivity chemiluminescent imaging system (model: Chemi DocTM XRS+) was purchased from Bio-Rad (Shanghai, China). Automatic chemiluminescent image analysis system (model:Tanon-5200)was purchased from Tanon Science & Technology (Shanghai, China).

2.3. Animal modeling

Body weight, toe temperature, rectal temperature, and spontaneous activity from each experimental group were assessed before

modeling, and blood was collected from the tail vein to measure of ACTH, CORT, and CRH by enzyme-linked immunosorbent assay (ELISA). The model group animals were intramuscularly injected with hydrocortisone solution (25 mg/kg, 500 mg hydrocortisone+1 ml DMSO+8 ml PEG300 + 1 ml Tween-80 + 10 ml NS) in the hind limbs for 21 consecutive days, once a day, alternating between the left and right hind limbs, the blank group rats were injected with normal saline. Body weight, toe temperature, rectal temperature, spontaneous activity, and ELISA assays were performed again to screen for successfully modeled animals, successfully modeled animals exhibited decreased body weight, toe temperature, anal temperature, and spontaneous activity, and decreased serum ACTH, CORT, and CRH hormone levels, and those thus identified underwent long-snake moxibustion and other interventions.

2.4. Intervention with long-snake moxibustion and medication

The rats were divided into a blank group (n = 6), a model group (n = 6), a long-snake moxibustion group (n = 6), a sham intervention group (n = 6), and a 11β-HSD1 inhibitor (BVT2733) + long-snake moxibustion group (n = 6). After shaving the back of the rats in each group, rats from the model and blank groups were fixed on a rat fixator for approximately 0.5 h every two days, whereas those in the long-snake moxibustion group received long-snake moxibustion treatment after being fixed on the rat fixator with the same frequency. The long-snake moxibustion procedure was carried out in the following manner: a 0.4 cm wide and 0.3 cm high layer of ginger paste was applied to the rats' Governor Vessel, where a strip of moxa was placed and ignited. The treatment was deemed complete when the moxa strip had burned out. Each session consisted of five moxa applications, and the entire procedure involved seven long-snake moxibustion treatments in total, treatments are conducted between 8a.m and 12p.m. In the sham intervention group, the rats were fixed in the same manner as the long-snake moxibustion group but the moxa strip was not ignited. Rats from the BVT2733 inhibitor + long-snake moxibustion group were fixed and treated using the same protocol as that for the long-snake moxibustion group, and were administered 10 mL/kg of BVT2733 solution by gavage daily for 15 days. The timeline of the whole experiment is shown in Fig. 1.

2.5. Behavioral testing

2.5.1. Toe temperature and anal temperature detection

A temperature probe coated with vaseline was inserted approximately 3 cm into the rectum of the animal to measure rectal temperature. The temperature was read when the thermometer displayed stable numbers with a beeping sound, and the average of three measurements was recorded. A handheld infrared body surface thermometer was used to measure the temperature of the palm of the right forelimb, and the average of three measurements was recorded. Each group was assessed in rotation.

2.5.2. Spontaneous activity frequency detection

The rats were placed in an apparatus designed to measure spontaneous activity, with a detection time set for 13 min, including a 3 min adaptation period. Following this initial phase, the activity trajectories were documented for the next 10 min. The frequency of spontaneous activities was then calculated.

2.6. ELISA detection

The levels of ACTH, CORT, and CRH in the serum of the rats were measured using ELISA kits.

2.7. Immunohistochemistry

Liver tissue sections were prepared through baking, dewaxing, and rehydration. Antigen retrieval was performed using citric acid buffer, followed by 5 % BSA antigen blocking. Sections were incubated with the 11β-HSD1 primary antibody (1:100) at 4 °C overnight and subsequently with horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1:100). After DAB staining, the sections were counterstained with hematoxylin and eosin, dehydrated to transparency, sealed, and mounted. Observations were made conducted using an optic microscope (BX43, Olympus).

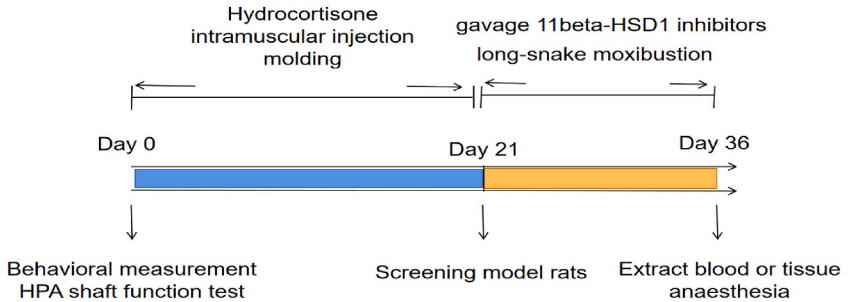

Fig. 1. Timeline of the experiment.

2.8. Western blot detection

A certain mass (50–100 mg)of rat liver tissue was collected and lysed using a lysis buffer radioimmunoprecipitation assays. The tissue was ground using a tissue grinder to extract total proteins. The lysate was centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatant was collected. A BCA protein quantitation kit was used to quantify total proteins. Denatured protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for 1.5 h, followed by transfer to polyvinylidene difluoride (PVDF) membranes at a constant current of 300 mA for 1h. After being blocked with a skimmed milk solution, the membranes were incubated with primary antibodies at 4 °C overnight, followed by incubation with secondary antibodies at room temperature for 2h. The PVDF membranes were soaked in the luminescent solution and imaged using an ultra-sensitive chemiluminescence imaging system.

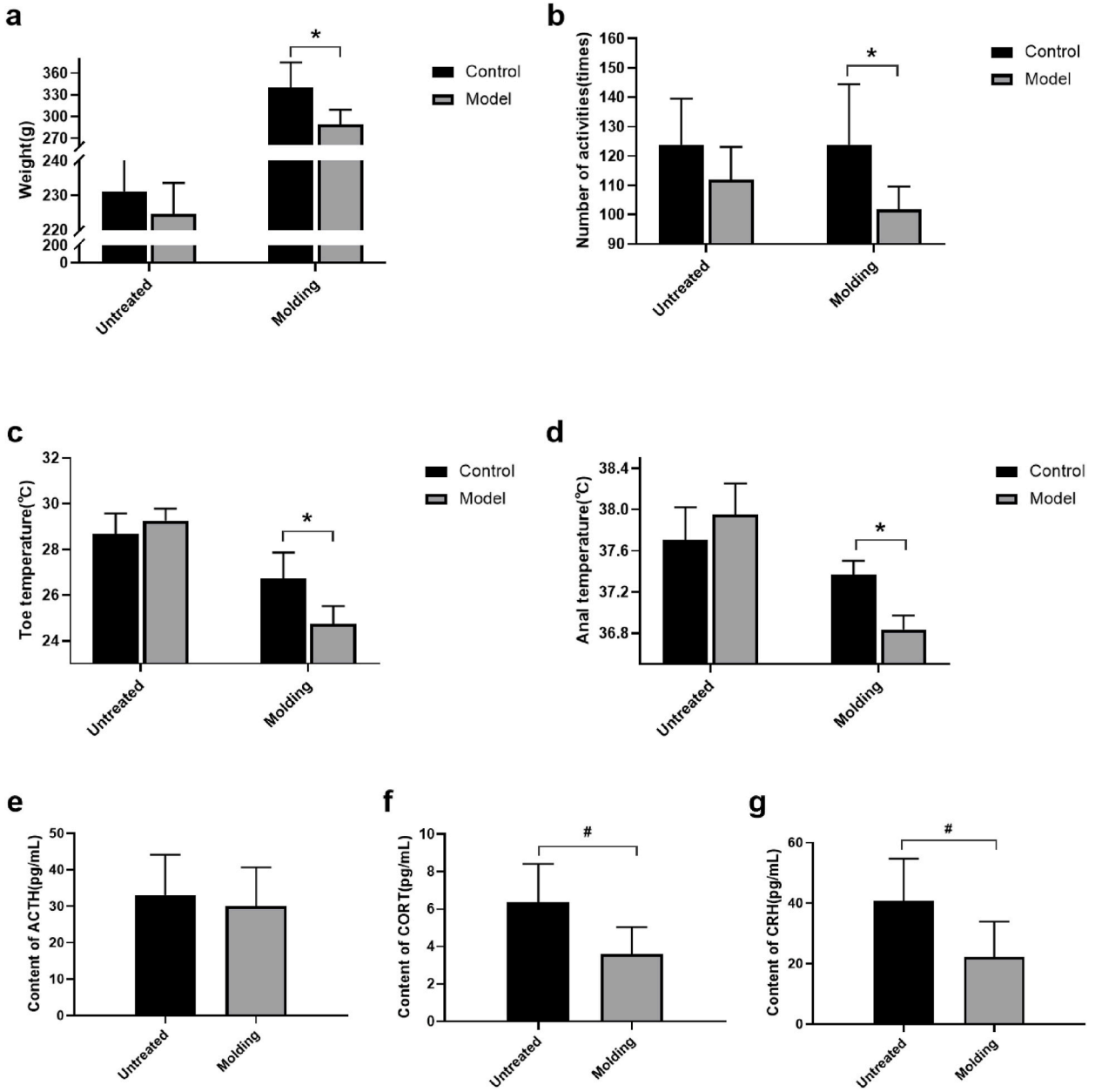

Fig. 2. Successful establishment of the KYDS rat model. Body weight (2a), spontaneous activity (2b), toe temperature (2c), and rectal temperature (2d) of rats in the control and model groups before and after modeling. *P < 0.05 compared with the control group; Levels of ACTH (2e), CORT (2f), and CRH (2g) in the serum of rats after modeling. $^{\#}P < 0.05$ compared with pre-modeling levels.

2.9. Statistical methods

All experimental data were analyzed using the Social Science Statistics Software Package (SPSS 25.0, Chicago, USA). When the data were normally distributed, the mean ± standard deviation ($\bar{x} \pm s$) was used to express the difference between groups, and one-way analysis of variance (ANOVA) was used for comparisons between groups. The least significant difference (LSD) test was used for pairwise comparisons. When data were skewed, the median and interquartile range were used to express the difference between groups, and the Rank Sum test was used for group comparisons. The significance level α was set at 0.05, and statistical significance was set at $P < 0.05$.

3. Results

3.1. Development of a KYDS rat model with low HPA axis function by intramuscular injection of hydrocortisone

After model establishment, rats in the model group exhibited a series of typical symptoms of KYDS, including anorexia, emaciation, disheveled and dull fur, hunched back, slow response, sluggish movement, stools that were clear and sparse, and increased urination. In addition, the body weight, toe temperature, rectal temperature, and spontaneous activity were measured before and after modeling, as shown in Fig. 2(a–d). There were no significant differences in terms of body weight, toe or rectal temperature, or spontaneous activity between the control and model groups before modeling; however, after modeling, the body weight, toe and rectal temperature, and spontaneous activity of the rats in the model group decreased significantly compared to the control group, with statistically significant differences ($P < 0.05$). This preliminary finding suggests that the rat model was successfully established.

The decline in HPA axis function and the reduced levels of ACTH, CORT, and CRH are important indicator for determining the

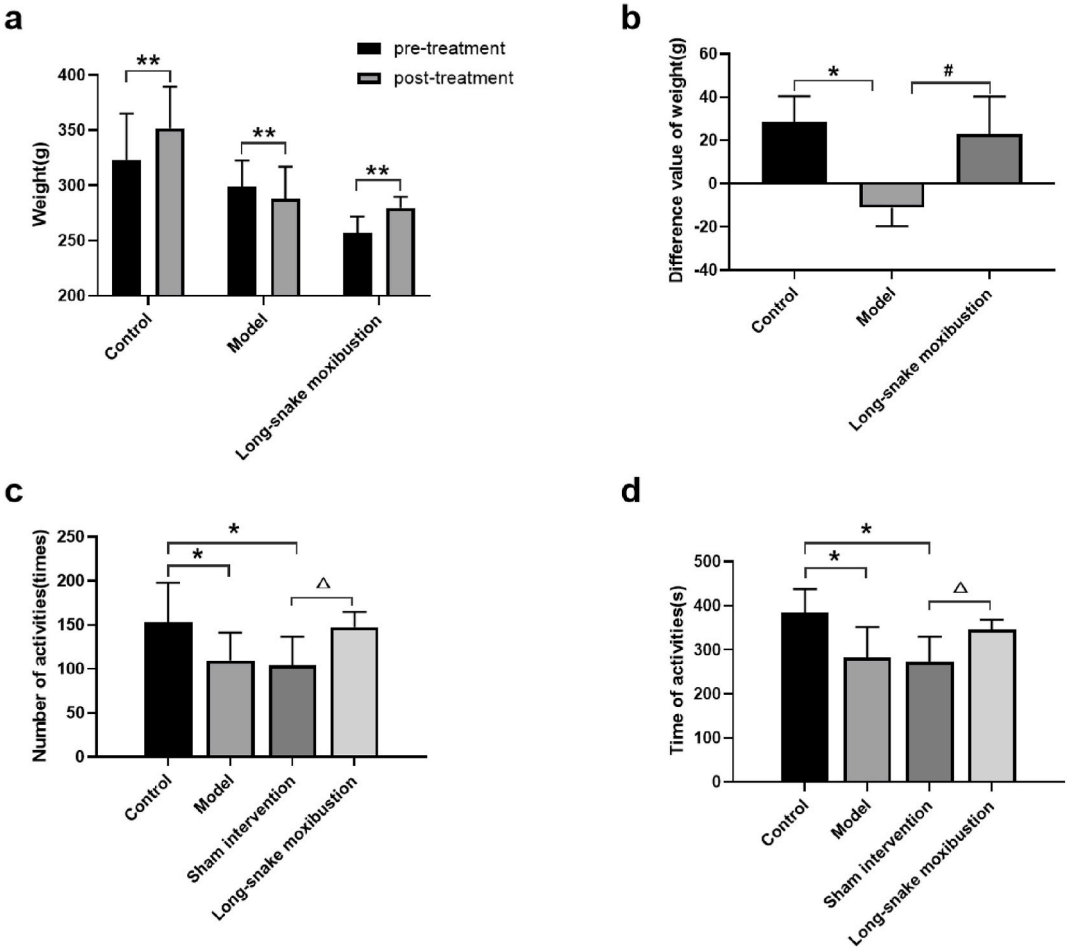

Fig. 3. Long-snake moxibustion improved physical signs in the rat model for KYDS. (a) Body weight of each group before and after treatment; (b) Weight differences before and after treatment for each group; (c) Activity Frequency of spontaneous activity in rats from each group after treatment; (d) Duration of spontaneous activity in rats from each group after treatment. $^{**}P < 0.01$ compared to before treatment; $^{*}P < 0.05$ compared to the blank group; $^{\#}P < 0.05$ compared to the model group; $^{\triangle}P < 0.05$ compared to the sham intervention group.

success of KYDS modeling in the rats. The results, as shown in Fig. 2(e–g), revealed that, compared to the pre-modeling levels, the levels of ACTH, CORT, and CRH had decreased after modeling. Although there was no significant difference in ACTH levels before and after modeling ($P > 0.05$), the differences in CORT and CRH levels were statistically significant ($P < 0.05$), indicating the successful development of a rat model for KYDS with impaired HPA axis function through the muscle injection of hydrocortisone.

3.2. Long-snake moxibustion improved physical signs of rats in the KYDS group

Rats underwent long-snake moxibustion treatment once every other day for a total of seven treatments after model establishment. Body weight measurements were taken for each group of rats before and after treatment, as shown in Fig. 3(a and b). The body weight of rats in the blank and long-snake moxibustion groups increased significantly after treatment, while the body weight of rats in the model group continued to decrease ($P < 0.05$). Comparing the weight differences before and after treatment among groups, we found significant differences between the blank and model groups, and between the model and long-snake moxibustion groups ($P < 0.05$). The effect of long-snake moxibustion on spontaneous activity frequency and duration are depicted in Fig. 3(c and d). The frequency and duration of spontaneous activity in the model and sham intervention groups were significantly reduced ($P < 0.05$), while those of rats in the long-snake moxibustion group increased compared to the model and sham intervention groups, with a significant difference compared to the sham intervention group ($P < 0.05$) but no significant difference compared to the blank group ($P > 0.05$).

3.3. Long-snake moxibustion increases 11β-HSD1 expression and plays a role in regulating the function of the HPA axis

We examined the expression of 11β-HSD1 in the livers of rats from each group using immunohistochemistry and Western blotting. As shown in Fig. 4(a and b), the immunohistochemistry results revealed that the level of 11β-HSD1 in the livers of rats from the model group significantly decreased compared to that in the blank group, and its activity was reduced. In contrast, the expression of 11β-HSD1 in the livers of rats from the long-snake moxibustion group increased compared to that in the model group. As shown in Fig. 4(c and d), the Western blotting results revealed that the protein expression level of 11β-HSD1 in the livers of rats from the model group significantly decreased compared to that in the blank group ($P < 0.05$). In contrast, the protein expression of 11β-HSD1 in the livers of rats from the long-snake moxibustion group increased compared to that in the model group ($P < 0.05$). This indicates that 11β-HSD1 expression is reduced in the liver of the rat model for KYDS and that long-snake moxibustion can restore the reduced 11β-HSD1 content and enhance its activity.

In addition, to explore the role of 11β-HSD1 in long-snake moxibustion's regulation of the HPA axis in our rat model, we used ELISA to determine the content of ACTH, CORT, and CRH in the serum of rats from each experimental group. As shown in Fig. 5, compared

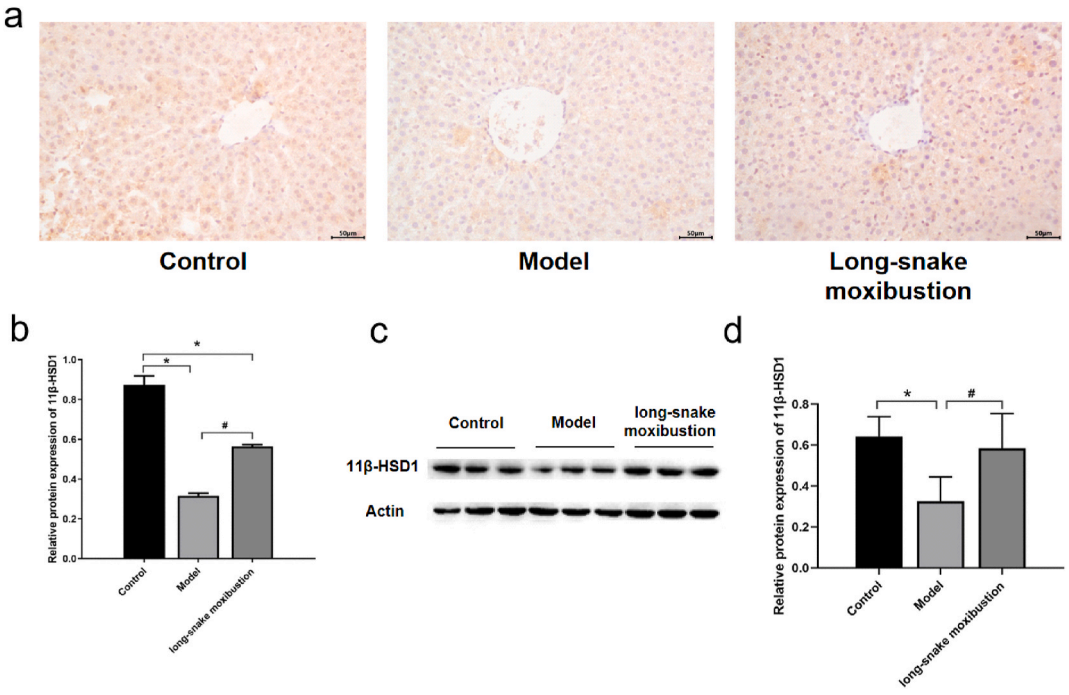

Fig. 4. Long-snake moxibustion upregulates the expression of 11β-HSD1 in the rat model for KYDS. (a) Representative immunohistochemistry images from each experimental group, scale bar 50 μm; (b) Immunohistochemistry results showing the liver content of 11β-HSD1 in each group; (c) Representative Western blot images from each group; (d) Western blotting results showing the liver content of 11β-HSD1 in each group. $^{*}P < 0.05$ compared with the blank group; $^{\#}P < 0.05$ compared with the model group.

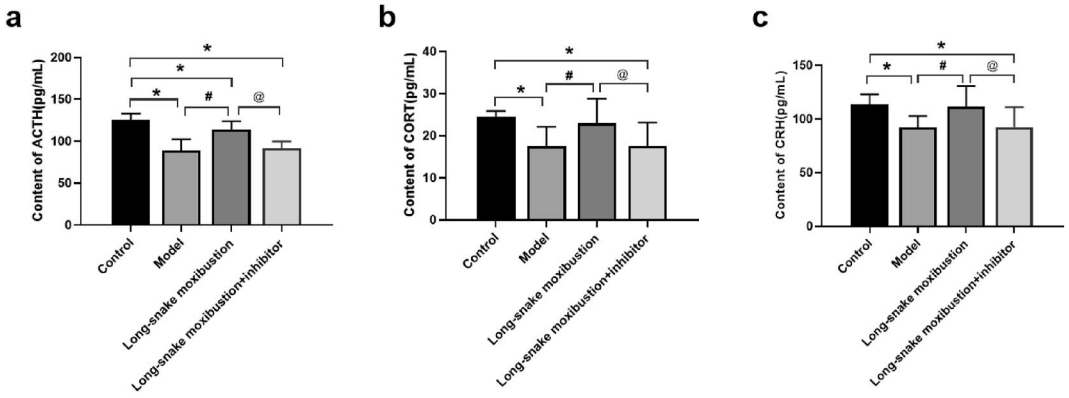

Fig. 5. Long-snake moxibustion regulates the HPA axis in the rat model for KYDS through 11β-HSD1. Changes in ACTH, CORT, and CRH expression after treatment in each group. (a) ACTH; (b) CORT; (c) CRH. $^{*}P < 0.05$ compared with the control group; $^{\#}P < 0.05$ compared with the model group; $^{@}P < 0.05$ compared with the long-snake moxibustion group.

with the blank group, ACTH, CORT, and CRH levels in rats from the model group decreased (all $P < 0.05$); and compared with the model group, ACTH, CORT, and CRH levels in the long-snake moxibustion group were significantly increased (all $P < 0.05$). Compared with the long-snake moxibustion group, ACTH, CORT, and CRH levels in the long-snake moxibustion +11β-HSD1 inhibitor group decreased (all $P < 0.05$). Long-snake moxibustion can increase the content of ACTH, CORT, and CRH in the serum, regulate the HPA axis in rats, and is closely related to the expression of 11β-HSD1.

3.4. 11β-HSD1 reductase activity mediates the regulation of the rat HPA axis by long-snake moxibustion

11β-HSD1 primarily exhibits high reductase activity in vivo, which is dependent on hexose-6-phosphate dehydrogenase (H6PDH) and related to NAPDH. To investigate the role of 11β-HSD1 reductase activity in the regulation of the HPA axis in our model by long-snake moxibustion, the protein expression levels of H6PDH and NAPDH in the experimental groups were detected by Western blotting. As shown in Fig. 6, compared with the control group, the expression levels of H6PDH and NAPDH in the livers of rats from the model group significantly decreased (both $P < 0.05$). In addition, compared with the model group, the expression levels of H6PDH and NAPDH in the long-snake moxibustion group significantly increased (both $P < 0.05$).

4. Discussion

TCM emphasizes that the kidneys are the foundation of innate vitality. Kidney yang, as the primary driving force of kidney essence, is responsible for warming, securing, and transforming [17]. Weakness in kidney yang leads to insufficient life vitality, manifesting as symptoms such as soreness and weakness of the lower back and knees, aversion to cold with cold limbs, loose stools, frequent clear urination, pale tongue with white coating, and deep, weak pulse [18]. These symptoms collectively constitute one of the common clinical patterns in Chinese medicine known as KYDS. Prolonged yang deficiency disrupts the balance of yin and yang within the body, reducing its defensive capabilities and increasing susceptibility to illness [19].

Modern research has found a close association between Kidney Yang Deficiency and dysfunction of the HPA axis, as well as decreased function of target glands such as the adrenal glands and thyroid [20]. The HPA axis is a crucial endocrine regulatory system

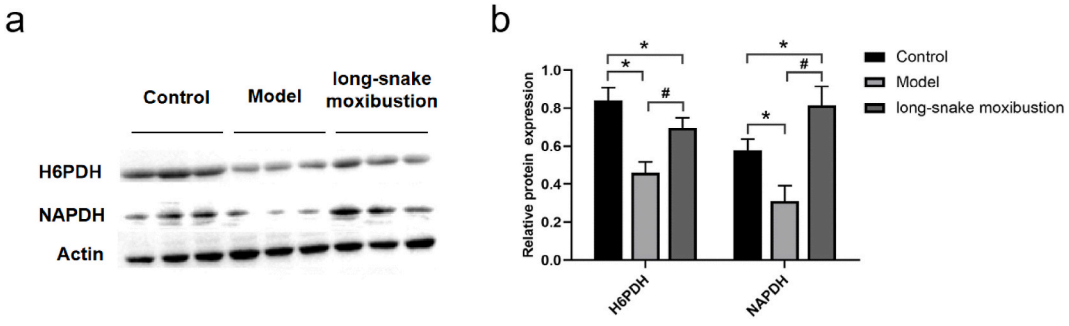

Fig. 6. Long-snake moxibustion enhances 11β-HSD1 reductase activity. Expression levels of H6PDH and NAPDH after treatment in each group. (a) Representative Western blot images for each experimental group; (b) Western blot results showing the content of H6PDH and NAPDH in the liver of rats from each group. $^{*}P < 0.05$ compared with the blank group; $^{\#}P < 0.05$ compared with the model group.

in the human body, governing the secretion and metabolism of various hormones. Kidney Yang Deficiency may lead to deterioration or imbalance in these glandular functions, thereby impacting hormone levels and metabolic processes within the body. Specifically, hormones like adrenaline and thyroxine are vital for maintaining energy metabolism, nervous system regulation, and immune function. Kidney Yang Deficiency could result in reduced or unstable secretion of these hormones, affecting overall metabolic status and stress responses. Furthermore, changes in HPA axis function are closely related to stress responses and emotional regulation. Kidney Yang Deficiency may disrupt normal HPA axis regulation, contributing to emotional fluctuations, increased fatigue, and other psychological and physiological responses [21]. Therefore, in-depth exploration of the relationship between Kidney Yang Deficiency and HPA axis function could enhance our understanding of the relevance of TCM theories on kidney deficiency in modern medicine, potentially offering new avenues for the prevention and treatment of conditions associated with Kidney Yang Deficiency.

KYDS is the earliest established TCM syndrome model. Currently, there are various methods for constructing the KYDS model, including drug-induced modeling, surgical modeling, and TCM etiological modeling [22]. Hydrocortisone administration is the most classic and widely used drug-induced modeling method for KYDS, which can be performed via intraperitoneal or intramuscular injection, or through gavage [23]. In our preliminary experiments, we found that rats injected with either hydrocortisone solution or hydrocortisone suspension by intraperitoneal injection experienced symptoms such as coma, limb paralysis, intestinal adhesion, and intestinal obstruction, with a high mortality rate (67.5 %, 27/40). This may have been related to the content of the drug and ethanol in the reagent and to the method of administration [24,25]. Intraperitoneal injections are rapidly absorbed into the bloodstream via the peritoneal membrane, resulting in a fast onset and significant effects. In contrast, gavage administration leads to slower absorption and reduced efficacy due to the metabolism in the gastrointestinal mucosa and liver. Therefore, we chose intramuscular injection in this study as the method of administration to establish a KYDS model. We found that the model rats exhibited hunched backs, slow reactions, sparse and dull fur, and reduced food and water intake after modeling, and their general condition and physical signs, such as body weight, toe temperature, rectal temperature, and spontaneous activity frequency, significantly decreased, consistent with the findings of previous studies [26,27]. In addition, hydrocortisone, as an exogenous glucocorticoid, can inhibit ACTH release when administered in large doses, further reducing adrenal cortical secretion of CORT and causing HPA axis dysfunction [28,29]. We also found that the levels of ACTH, CORT, and CRH in the model rats significantly decreased after modeling. This result aligns well with the research objective of exploring the molecular mechanisms by which long-snake moxibustion affects the HPA axis in rats with KYDS. Using this model for our research and conducting a series of subsequent studies enhanced the feasibility and stability of this study.

Moxibustion has beneficial effects, including anti-inflammatory, tissue repair, and regulatory properties, which can increase the number of cells in the body, enhance cellular phagocytic function, regulate the activity of non-specific immune substances, and improve the body's overall immune capacity [30,31]. Long-snake moxibustion, also known as Du moxibustion or extending moxibustion, is a TCM technique that involves application of thick ginger to the patient's spinal area, placing moxa atop the ginger, and igniting the moxa. Among the various moxibustion methods, long-snake moxibustion has the most potent warming effect, the largest treatment area, and the longest duration. It has demonstrated significant therapeutic benefits for clinical deficiency cold-type diseases, such as Yang deficiency with infections [7], lower back pain [10], and irritable bowel syndrome [8]. Existing evidence suggests that moxibustion can effectively regulate the levels of CORT, ACTH, and other substances in rats with irritable bowel syndrome, restoring the negative feedback function of the HPA axis [32]. Our previous study found that long-snake moxibustion could reduce the Yang deficiency score, improve symptoms such as cold intolerance in individuals with Yang deficiency, increase serum levels of ACTH, CORT, and CRH, and regulate the function of the HPA axis [11]. This prompted our in-depth investigation into the molecular mechanisms of long-snake moxibustion in regulating the HPA axis in individuals with Yang deficiency. In the present study, Long-snake moxibustion treatment was shown to improve physical signs such as emaciation, reduced activity, and slow movement in our rat model of KYDS, restoring their Yang deficiency state and raising ACTH, CORT, and CRH levels. These findings are consistent with previous research by Min et al. [33] and Ren et al. [34].

Long-snake moxibustion can increase the expression of 11β-HSD1. After administering an 11β-HSD1 inhibitor, the regulatory effect of long-snake moxibustion on the HPA axis weakened as 11β-HSD1 levels decreased, suggesting that 11β-HSD1 may play a crucial role in regulating the HPA axis in our rat model through long-snake moxibustion. Previous studies also observed that mRNA and protein expression of 11β-HSD1 in the amygdala and hypothalamus of kidney-deficient rats significantly increased after moxibustion compared to the model group [35]. 11β-HSD is a crucial enzyme for glucocorticoid metabolism and comprises two isomers: 11β-HSD1 and 11β-HSD2. This enzyme exhibits bidirectional activity, functioning as both a dehydrogenase and a reductase, with the reductase function being predominant in vivo. It converts inactive dehydrocorticosterone into active corticosterone, thereby increasing tissue glucocorticoid levels. In contrast, 11β-HSD2 inactivates corticosterone to dehydrocorticosterone and is primarily found in the kidneys [36]. 11β-HSD1 activity significantly impacts HPA axis function due to its expression location and its influence on local glucocorticoid and CORT concentrations. Previous studies have shown that overexpression of 11β-HSD1 in SCP-1 cells increases extracellular CORT levels approximately 3.3 times compared to wild-type cells. Conversely, knocking out 11β-HSD1 results in nearly undetectable CORT production in the cells [37]. Furthermore, in the pituitary of mice with chronic arthritis, the upregulation of 11β-HSD1 and the enhancement of glucocorticoid negative feedback may be key factors contributing to abnormal HPA axis activity [38]. The co-localization of 11β-HSD1 and CRH in the paraventricular nucleus further supports their role in regulating the HPA axis [39].

H6PDH is a glucose dehydrogenase located in the lumen of the endoplasmic reticulum. In mature cells, the activity of 11β-HSD1 is primarily regulated by H6PDH, which plays a crucial role in maintaining 11β-HSD1 reductase activity and mediating local glucocorticoid production [40]. The cofactor for H6PDH is NADP+, and its substrate is glucose-6-phosphate (G6P). H6PDH converts G6P into 6-phosphogluconate and generates NADPH, altering the ratio of NADP+/NADPH and enabling 11β-HSD1 to function as a reductase [41]. Previous studies have shown that in transgenic mice lacking H6PDH, the reductase activity of 11β-HSD1 decreases, resulting in a predominant dehydrogenase activity [42,43]. These findings suggest that the reductase activity of 11β-HSD1 depends on

the involvement of H6PDH and NADPH. In this study, we aimed to investigate the role of 11β-HSD1 reductase activity in regulation HPA axis function in Yang deficiency rats through long-snake moxibustion. We measured the protein levels of H6PDH and NADPH and found that long-snake moxibustion increases the expression of both H6PDH and NADPH, thereby enhancing the reductase activity of 11β-HSD1.

This study has some limitations. We did not administer the 11β-HSD1 inhibitor to a separate control group of rats to compare the results with those from the long-snake moxibustion group. As a result, we cannot determine the potential relationship between these two experimental conditions. Future research could address this limitation by exploring the effect of long-snake moxibustion as a potential 11β-HSD1 inhibitor. This would involve treating one group of rats with the 11β-HSD1 inhibitor alone and another group with long-snake moxibustion alone. Comparing the results from these two groups would provide a clearer understanding of the specific effects of long-snake moxibustion on 11β-HSD1 inhibition. Additionally, there are differences between the animal model used for KYDS and clinical cases of Yang deficiency. Therefore, future clinical trials with appropriate design should be conducted to evaluate the regulatory effect of long-snake moxibustion on 11β-HSD1 function in patients with Yang deficiency. Overall, these findings suggest that long-snake moxibustion can regulate symptoms and HPA axis function in a rat model of KYDS, potentially mediated by increased expression and reductase activity of 11β-HSD1 in the liver.

5. Conclusions

In conclusion, KYDS, as the earliest established TCM syndrome model, has been approached with various modeling methods, including the administration of hydrocortisone. In this study, we opted for intramuscular hydrocortisone injections, observing a range of symptoms of Yang deficiency and HPA axis dysregulation in rats. Moxibustion, as a traditional Chinese technique, significantly ameliorated these symptoms and physiological markers by modulating 11β-HSD1 expression and activity. While this study has its limitations, future research could further explore moxibustion's potential as an 11β-HSD1 inhibitor and validate its regulatory mechanisms in Yang deficiency patients through clinical trials. These findings not only deepen our understanding of TCM treatments for KYDS models but also offer new directions and insights into treating Yang deficiency-related disorders.

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Ethical statement

The Jiangxi Zhonghong Boyuan Biotechnology Co., Ltd. Ethics Committee examined and approved the study scheme (ethics approval number 2020083001).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Hui Huang: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Jingjiao Zeng:** Writing – original draft, Visualization, Formal analysis. **Limei Tang:** Writing – original draft, Visualization, Formal analysis. **Lele Geng:** Data curation, Conceptualization. **Xijing Yu:** Data curation, Conceptualization. **Chenying Deng:** Writing – review & editing. **Hang Liu:** Writing – review & editing. **Ping Huang:** Visualization. **Ensi Hong:** Visualization. **Xiuwu Hu:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38486.

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