Original Article

Effects of decoction of *Angelica Sinensis, Zingiberis Rhizoma Recens*, and mutton on physiology and biochemistry of Sprague Dawley female rats with spleen-kidney Yang deficiency

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Background: To examine the effects of each dose of decoction of *Angelica sinensis* (Dang gui), *Zingiberis Rhizoma Recens* (Sheng jiang), and mutton (DAZM) on the physiological and biochemical indexes of female rats with spleen-kidney Yang deficiency (SKYD) through 30-day feeding of DAZM, and to evaluate the tonifying effect of DAZM combined with the system of benefit damage index-general score (BDI-GS).

Methods: Sprague Dawley (SD) rats were administered adenine and senna water to establish a SKYD model. The rats were then allocated to 4 groups at random: Model group, and L group, 4.2 g/kg, M group, 8.4 g/kg and H group, 16.8 g/kg. In addition, the group of normal feeding with unlimited diet was set as N group. Blood samples were taken to detect the relevant physiological and biochemical indexes. For organ coefficient analysis, 10 kinds of organ tissues were dissected and weighed. The tonifying effect of DAZM was discussed according to the BDI-GS system.

Results: During the modeling, the weight of rats in the normal group displayed a marked growth trend, and the weight of the model group was markedly lower than that of the normal group. After feeding the rats DAZM at a low, intermediate, and high dose, the anal temperature of rats in each group continued to rise, and finally remained basically the same as that of normal rats. Hematological and urine examinations revealed that the urea nitrogen and creatinine (CRE) of the model group and the experimental group were markedly higher than those of the normal group, and there were marked differences. After intragastric administration of DAZM, E2 increased markedly. The BDI-GS values of the liver, spleen, lung, kidney, brain, ovary, and adrenal gland of female rats in the 3 administration groups of DAZM were >1, and the total cumulative GS value of each organ of female rats was more than 10.

Conclusions: The decoction of DAZM has no obvious effect on the growth, metabolism, and development of female rats with SKYD, causes no obvious damage to organs, and has a certain reparative effect on the kidney damage caused by SKYD.

Keywords: Decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton (DAZM); spleen-kidney Yang deficiency (SKYD); effects of physiology and biochemistry; benefit damage index-general score (BDI-GS)

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Introduction

Decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton (DAZM), derived from Zhang Zhongjing's Synopsis of the Golden Chamber, who is heralded as the medical saint of the Han Dynasty, is a pioneering famous prescription for food therapy and medicine, which has the functions of warming and tonifying insufficiency, dispersing cold, and relieving pain (1). In China, it is a popular soup, which is especially suitable for people with deficiency of spleen-kidney Yang deficiency (SKYD) (2); such people often have cold hands and feet and an aversion to cold. Traditional Chinese medicine (TCM) theory states that "Yang" and "Yin" are opposite, yet mutually engendering forces. The propensity of Yang is upward and outward movement; it is the root of human life activities and the source of transforming materials (3,4). Yang-qi has the function of strengthening and protecting, which is mainly embodied in 2 aspects: one is that Yang-qi protects the body surface against the invasion of diseases and pathogens, the other is that Yang-qi can stabilize and control fine substances such as blood and cellular fluid to prevent accidental loss. The body temperature is constant, which requires the maintenance of Yang-qi. The physiological activities of the internal organs and meridians can only be carried out under the warming effect of Yang-qi. If Yang-qi is insufficient, Yin-cold prevails endogenously, and fluid and blood become stagnant without adequate flow. The situation of SKYD leads to digestive dysfunction and disordered water metabolism, Such as intestinal stress syndrome, inflammatory bowel disease, dysmenorrhea. Its clinical symptoms include pale complexion, chilly limbs, cold pain in the waist and knees or lower abdomen, chronic diarrhea and dysentery, or diarrhea between 5:00 am and 18:00 pm, diarrhea with undigested food, or poor urination, swollen face, and so on. According to the works—Synopsis of the Golden Chamber: Treatment of Abdominal Fullness, Cold Hernia, Persistent food disease and Pulse syndrome, for those with cold hernia, abdominal pain, and hypochondriac pain, DAZM is the main choice as a food therapy and medicine, and in the works—Synopsis of the Golden Chamber: Pulse syndrome of postpartum disease in women, "Postpartum abdominal pain should be treated with DAZM; at the same time, the decoction could treat abdominal cold hernia, and insufficiency of fatigue" (5). The original prescription consists of 45 g Angelica sinensis, 250 g mutton, and 75 g *Zingiberis Rhizoma Recens*, all of which are listed as Chinese medicine for both medicine and food by the National Health Commission (6). *Angelica sinensis* replenishes and invigorates blood, *Zingiberis Rhizoma Recens* warms the middle burner and dispels cold, and mutton is a product of flesh and blood, which can replenish insufficiency and dispel cold (7), mainly indicated for colic and abdominal pain with cold and blood insufficiency, as well as women's dysmenorrhea and postpartum conditioning (6,8-11).

In this study, DAZM was used as the test object to examine the clinical features of rats in the 30-day feeding experiment of DAZM. Combined with the benefit damage index-general score (BDI-GS) evaluation system, BDI: the mean value of the statistical value of the organ index of the test object/the mean value of the corresponding index of the control group, GS: based on the BDI value. The tonifying effect of DAZM on Sprague Dawley (SD) female rats was evaluated by cumulative score as the evaluation

Highlight box

Key findings

- The decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens* , and mutton (DAZM) has no obvious effect on the growth, metabolism, and development of rats with spleen-kidney Yang deficiency (SKYD), causes no obvious damage to organs, and has a certain reparative effect on the kidney damage caused by SKYD.

What is known and what is new?

- Through the modification of physiological and biochemical indexes of feeding test, combined with the benefit damage index-general score (BDI-GS) index system, the results suggest that DAZM has a certain tonifying effect on female rats with SKYD.
- DAZM can elevate rectal temperature and affect the cAMP/cGMP level. DAZM can restore the anal temperature of SKYD to normal.

What is the implication, and what should change now?

- DAZM has no obvious effect on the growth, metabolism, and development of female rats with SKYD, causes no obvious damage to organs, and has a certain reparative effect on the kidney damage caused by SKYD.
- DAZM increases body temperature by changing cAMP/cGMP levels.

index of overall comprehensive benefits and health effects (12-17). Tonifying effect refers in the TCM theory is that Chinese medicine can tonify the body material loss, enhance the function of human activity, improve disease resistance, eliminate weak meaning. Similar to the modern pharmacology that several cases are tonify effect: one is the non-specific immune function and specific immune function are enhanced, the performance can improve the body's adaptability, enhance the body 's resistance to various harmful stimuli, regulate the pathological process, so that the function of the disorder returned to normal. Also such as tonic effect can promote the adrenal cortex system excitement, can promote breast, uterus, vagina, ovary, testis development. Regulation of glucose and fat metabolism, and can promote protein synthesis, increased albumin and globulin content, or refers to improve the body's ability to work, improve sleep and appetite, and can reduce fatigue, weight gain, nourishing strong role. The BDI and GS are combined to analyze the sub-acute experimental results of the test substance, and then evaluate the beneficial effect of the test substance, which has been explored and applied in recent years. Evaluating the physiological and biochemical effects of DAZM after long-term use could serve as scientific evidence for the comprehensive appliance of DAZM. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6442/rc).

Methods

Experimental animals

Since the spleen and kidney yang deficiency is more common in women, female rats were used to simulate human performance. The experimental animals were specific-pathogen-free (SPF) grade SD female rats provided by the Science and Technology Center for Experimental Animals of Jiangxi University of Chinese Medicine (CM). The weight range of rats was 180–220 g (8 week-age), and the certificate number was SCXK (GAN) 2018-0003. The experimental rats were fed in the SPF barrier system of the Science and Technology Center for Experimental Animals of Jiangxi University of TCM. The feeding environmental conditions were that the ambient temperature was 20–25 ℃, the humidity was 45–55%, and the license No was SYXK (GAN) 2017-0004. Animal experiments were performed under a project license (No. JZLLSC20210053) granted by experimental animal Ethics Committee of Jiangxi University of Chinese medicine, in compliance with Jiangxi University of Chinese medicine guidelines for the care and use of animals.

Reagents and instruments

The liver-kidney function kits were provided by Nanchang Tekang Technology Co., Ltd. (Nanchang, China), to measure the following: total protein (TP; batch number: 20210313), albumin (ALB; batch number: 20210311), alanine aminotransferase (ALT, batch number: 20210215), aspartate aminotransferase (AST; batch number: 20210216), total bilirubin (TB; batch number: 20210310), blood urea nitrogen (BUN; batch number: 20210308), uric acid (UA; batch number: 20210408), creatinine (CRE; batch number: 20210216), glucose (Glu; batch number: 20210325), total cholesterol (TC; batch number: 20210326), and triglyceride (TG; batch number: 20210328). Testosterone (T; batch number: 20210509), estradiol (E2; batch number: 20210510), adrenocorticotropic hormone (ACTH; batch number: 20210518), cyclic adenosine monophosphate (cAMP; batch number: 20210507), cyclic guanosine monophosphate (cGMP; batch number: 20210511), triiodothyronine (T3; batch number: 20210521), and thyroxine (T4; batch number: 20210504) kits were obtained from Shanghai Hepeng Technology Co., Ltd. (Shanghai, China).

Additional equipment used for the experiments included an automatic veterinary blood analyzer (Nanchang Tekang), automatic biochemical analyzer (Beckman Coulter, Brea, CA, USA), urine analyzer (Youlite, Guilin, China), low temperature and high-speed freezing centrifuge (Shanghai An Ting, Shanghai, China), ultra-high resolution microscope system (Leica, Wetzlar, Germany), automatic dehydrator (Leica), paraffin slicer (Leica), automatic dyeing and sealing workstation (Leica), and an automatic digital slice scanning and appliance system (Leica).

Molding

Adenine (batch No. 20210307) provided by Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China) and 100% Senna Decoction (batch No. 20210411) provided by Jiangxi Guhan Refined TCM Decoction Pieces Co., Ltd. (Jiangxi, China) were prepared to create an adenine solution with a concentration of 0.2 g/kg using adenine and distilled water, and the model of SKYD was induced by gavage with adenine and iced senna water.

Test substance

DAZM, according to "Golden Chamber (belongs to Canon on Source and Dose of Chinese Herbal Formulas)" written by Zhang Zhongjingm, TCM master of the Eastern Han Dynasty, is composed of 45 g of *Angelica sinensis*, 75 g of *Zingiberis Rhizoma Recens*, 250 g of mutton. The 3 herbs were placed in 1,600 mL of water, boiled down to 600 mL, and administered warm (140 mL each time).

Dose calculation and setting: the concentration of DAZM was 370 g (45+75+250)/600 mL =0.62 g/mL, and 140 mL is taken orally (per 60 kg), so the amount per kg was 2.3 mL, specifically, the amount per kg was 1.42 g/kg. According to the conversion coefficient of 60 kg of humans and rats of 6, the amount for rats according to the equivalent human dose was 8.4 g/kg. We set this amount as the middle dose of rats, set the low dose at half of this dose (i.e., 4.2 g/kg), and set the high dose at double the middle dose (16.8 g/kg).

Study methods

Modeling and animal grouping

Referring to the literature (18,19), the rat model of SKYD was established by using the compound method of adenine and iced senna water. The model evaluation was introduced in another paper. The experiment was carried out on the basis of this model according to the 30-day feeding test method specified in the 30- and 90-day feeding test (GB15193.13-2003). Rats were quarantined for 5 days. A total of 60 healthy and active rats with good nutrition were selected. Intake of adenine and Senna water by 48 female SD rats led to SKYD, and these rats were stochastically divided into 4 groups: model group (Model group, water, 0 mg/kg), low-dose group (L group, 4.2 g/kg), medium-dose group (M group, 8.4 g/kg), and high-dose group (H group, 16.8 g/kg), with 12 rats in each group. When animals appear weight loss, fur fluffy dull, afraid of cold piled up, body temperature decreased, suggesting that animals appear SKYD.In addition, the remaining 12 rats were fed normally with an unlimited diet to comprise the normal group (N group). The adenine and senna were administered by continuous intragastric administration for 30 days, and the N and M groups were fed with an unlimited diet.

Observation and index detection

General observation

The examiners observed and recorded the general performance, behavior, poisoning performance, and death of animals every day, weighed the feed and drinking water of each group, and examined the changes of daily food intake and drinking water. Rats were weighed once every other week and the changes of body weight and body temperature were examined every week.

Observation of hematological indexes and blood biochemical indexes

Abdominal aortic blood was taken for blood routine and biochemical detection, mainly including hemoglobin (Hgb), red blood cell (RBC) count, white blood cell (WBC) count and their classification. The automatic biochemical analyzer detected total TP, ALB, ALT, AST, TB, DB (direct bilirubin), BUN, UA, CRE, Glu, TC, and TG. The contents of T, E2, ACTH, cAMP, cGMP, T3 and T4 in blood were detected by enzyme-linked immunosorbent assay (ELISA).

Urinalysis

Guilin Youlite urine detection analyzer was used to analyze alkalinity (PH), urinary specific gravity (SG), urobilinogen (URO), occult blood (BLD), leukocyte (WBC), urinary protein (PRO), urinary sugar (Glu), bilirubin (BIL), ketone body (KET), urinary RBC, and urine color.

Observation of pathological indexes

The rats were killed after 2% pentobarbital anesthetization, the abdomen of the rats was opened, the thymus, heart, liver, spleen, lung, kidney, adrenal gland, uterus, and ovaries were harvested, and the visceral body ratio (organ coefficient) was calculated. Histopathological examination: the rats in each group were examined generally, and the liver, spleen, kidney, ovary, uterus, and brown fat were preserved for pathological section examination. They were fixed with 10% formaldehyde, embedded in conventional dehydration and paraffin, and then sliced (thickness 4 μm), dehydrated in gradient alcohol. Hematoxylin and eosin (HE) staining was performed after mild washing with distilled water, and the histopathological changes were examined under an optical microscope.

Data analysis and processing

BDI-GS evaluation method

The mean value of the statistical value of the organ index of the test object/the mean value of the corresponding index of N group was calculated to obtain BDI. On this basis, GS was obtained through cumulative integral calculation. The combination of BDI-GS was used as the evaluation index of the overall comprehensive benefits and health effects.

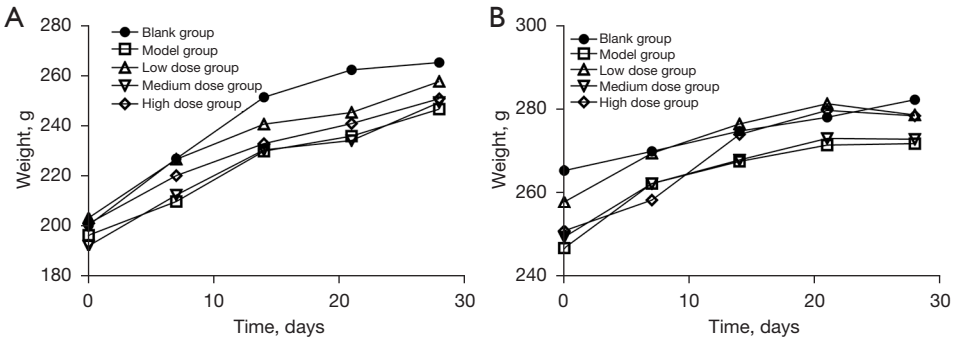

Figure 1 Weight change trend of female rats. (A) Modeling stage of SKYD. (B) After feeding DAZM. SKYD, spleen-kidney Yang deficiency; DAZM, decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton.

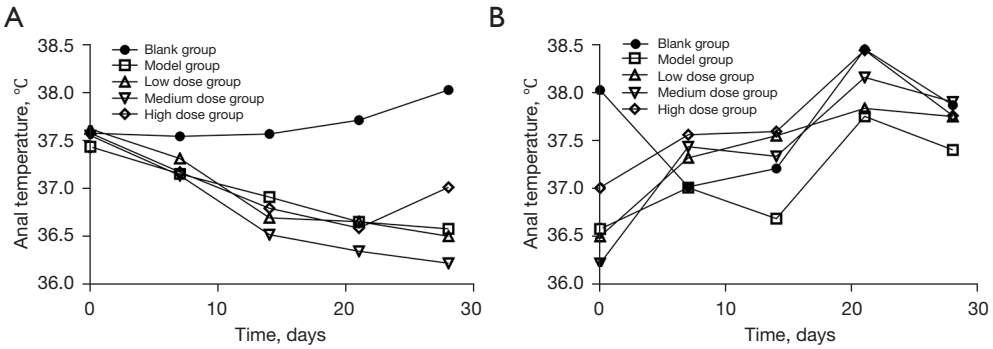

Figure 2 Change trend of anal temperature in female rats. (A) Modeling stage of SKYD. (B) After feeding DAZM. SKYD, spleen-kidney Yang deficiency; DAZM, decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton.

Statistical analysis

After BDI-GS calculation, the evaluation was repeated once in the same way, and similar repeatability evaluation results were obtained. The 2 groups of experimental data were combined for statistical analysis. The software SPSS 22.0 (IBM Corp., Armonk, NY, USA) was widely applied for statistical analysis. Data were expressed as ($\bar{x}\pm s$, n=12), and the marked differences were compared by paired sample *t*-test analysis between groups. GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) was used for visualization.

Results

Effect of DAZM on weight change of female rats with SKYD

Figure 1 shows the weight changes of female rats in the modeling stage of SKYD and the administration stage of DAZM. As shown in *Figure 1A*, the weight of rats in the normal group displayed a marked growth trend, and the weight of rats in the model group was markedly lower than that in the normal group. After feeding DAZM, the weight of rats was higher than that in the M group, but there was no marked difference (P>0.05), see *Figure 1B*.

Effect of DAZM on anal temperature in female rats with SKYD

Figure 2 shows the anal temperature changes of female rats in the modeling stage of SKYD and the administration stage of DAZM. As shown in *Figure 2A*, the anal temperature of normal rats in the modeling was basically stable and markedly higher than that of other groups, with marked difference (P<0.05). After feeding DAZM at low, intermediate, and high doses, the anal temperature of rats in each group continued to rise, and finally remained basically the same as that of normal rats. Although there was no marked difference between the dose groups (P>0.05), the anal temperature of the M group was markedly lower than

Table 1 Effect of DAZM on weekly drinking water of female rats with SKYD ($\bar{x}\pm s$)

Group	After modeling	7 d (g)	14 d (g)	21 d (g)	28 d (g)
N group	279.9±6.2	245.6±15.3	242.1±22.9	249.4±25.9	249.8±26.4
Model group	651.5±107.1*	755.9±30.2*	684.2±51.8*	666.9±42.2*	527.8±39.5*
L group	623.0±66.0*	715.7±29.8*	623.6±62.4*	598.5±52.0*	495.6±56.0*
M group	641.6±29.2*	774.8±28.2*	696.9±72.6*	675.2±62.7*	558.8±57.4*
H group	604.2±80.9*	807.1±30.4*	722.8±99.9*	684.1±86.2*	550.9±56.5*

*, in comparison with N group, P<0.05. DAZM, decoction of *Angelica sinensis, Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency; BW, body weight; N, normal group; Model, model control group; L, low dose group; M, medium-dose group; H, high dose group.

Table 2 Effect of DAZM on weekly food intake of female rats with SKYD ($\bar{x}\pm s$)

Group	After modeling	7 d (g)	14 d (g)	21 d (g)	28 d (g)
N group	191.9±35.2	188.8±12.6	186.1±20.9	178.5±18.2	176.8±15.6
Model group	165.0±53.0	213.3±11.3	207.2±13.6	183.0±12.4	187.3±14.8
L group	159.3±20.2	207.0±15.2	209.2±36.4	168.3±23.4	188.0±21.5
M group	152.6±24.8	192.5±21.9	189.1±19.6	168.9±23.1	173.4±21.1
H group	126.1±24.3*	173.1±18.7*	164.0±17.2*	143.6±19.7*	136.5±19.5*

*, in comparison with N group, P<0.05. DAZM, decoction of *Angelica sinensis, Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency; BW, body weight; N, normal group; Model, model control group; L, low dose group; M, medium-dose group; H, high dose group.

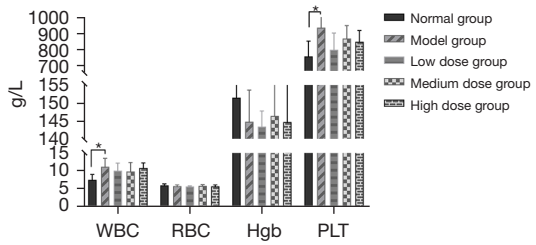

Figure 3 Effect of DAZM on hematological indexes of female rats with SKYD. *, in comparison with N group, P<0.05. WBC, white blood cell; RBC, red blood cell; Hgb, hemoglobin; PLT, platelet; DAZM, decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency.

the other groups (P<0.05), *Figure 2B*.

Effect of DAZM on the changes of drinking water and food intake of female rats with SKYD

Table 1 shows the effect of DAZM on the weekly drinking water volume of female rats with SKYD. The drinking water volume of the normal group was markedly lower than that of the 3 dose groups of DAZM and the Model group (P<0.05), whereas there was no marked difference between the 3 dose groups of the DAZM and Model group (P>0.05).

Table 2 shows the effect of DAZM on the weekly food intake of female rats with SKYD. The food intake of rats in the group of the high-dose DAZM was markedly lower than that in other groups (P<0.05), although there was no marked difference among the other groups (P>0.05).

Effect of DAZM on hematological indexes of female rats with SKYD

Figure 3 shows the effect of DAZM hematological indexes of female rats with SKYD. In comparison with the N group, the WBC and platelet (PLT) of female rats in the SKYD model group grew markedly (P<0.05). After feeding DAZM, the PLT decreased and tended to be normal, whereas there was no marked difference between the WBC of each dose group of DAZM and Model group (P>0.05); there was no marked difference in the contents of RBC and Hgb in each group.

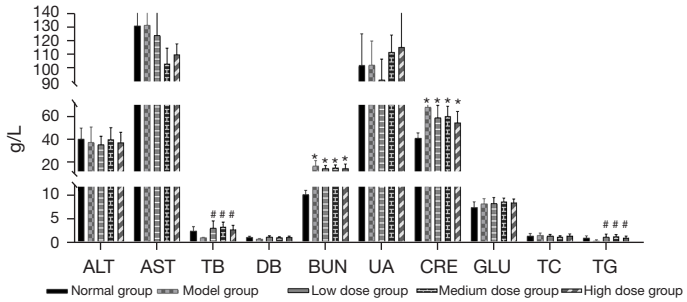

Figure 4 Effect of DAZM on physiology and biochemistry of female rats with SKYD. *, in comparison with N group, P<0.05; #, in comparison with Model group, P<0.05. ALT, alanine aminotransferase; AST, aspartate aminotransaminase; TB, total bilirubin; DB, direct bilirubin; BUN, blood urea nitrogen; UA, uric acid; CRE, creatinine; Glu, glucose; TC, total cholesterol; TG, triglycerides; DAZM, decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency.

Effects of DAZM on liver-Kidney function, blood lipid, and blood Glu of female rats with SKYD

Figure 4 shows the results of the end-stage biochemical tests of DAZM on female rats with SKYD. In comparison with the N and Model groups, the end-stage blood biochemical index test results of the 3 dose groups showed that the TB and TG of the female rats in the Model group were markedly lower than those in other groups, and there was a marked difference in comparison with the female rats in the 3 dose groups (P<0.05). The BUN and CRE of female rats in the Model group and the administration group were markedly higher than those in the N group (P<0.05). The BUN and CRE of the DAZM group showed a downward trend, but there was no marked difference in comparison with the model group (P>0.05). The indicators of ALT, AST, DB, TC, UA, and GLU had no marked impact between the groups.

Effect of DAZM on sex hormones in female rats with SKYD

The E2 level of the animal model of SKYD was markedly lower than that of N group (P<0.05). After feeding DAZM, the E2 level grew according to the dosage. There were marked differences between the intermediate and high dose group and Model control group (P<0.05), as shown in *Figure 5A*. In comparison with the N group, T in the Model group and DAZM decreased, but no marked difference was observed, as shown in *Figure 5B*.

Effects of DAZM on cAMP and cGMP in female rats with SKYD

Serum cAMP and cGMP not only coordinate with each other, but restrict and resist each other in terms of physiological effects *in vivo*. The content of cAMP is positively correlated with the growth of body temperature, and the content of cGMP is negatively correlated with the decrease of body temperature. After the model of SKYD, it can be seen that the levels of cAMP and cGMP decreased markedly. In comparison with N group (P<0.05). After feeding DAZM, cAMP grew with the growth of dose, and the difference between the intermediate and high dose levels and Model group was marked (P<0.05), whereas the content of cGMP changed little, as shown in *Figure 6*.

Effect of DAZM on T3 and T4 in female rats with SKYD

The T3 of rats with SKYD was markedly lower than that of those in the N group (P<0.05). After feeding DAZM, T3 showed an increasing trend with the growth of dose. There was a marked difference between the intermediate and high dose group and Model group (P<0.05), but there was no marked change in T4 between M group and each dose group and N group, as shown in *Figure 7*.

Effect of DAZM on urine of female rats with SKYD

Table 3 shows the effect of DAZM on the end-stage urine routine of female rats with SKYD. It can be seen from the

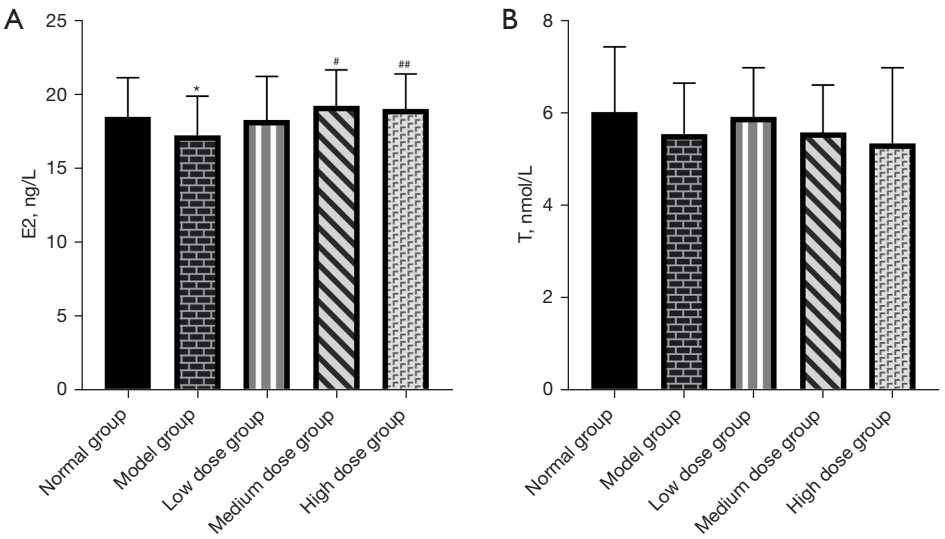

Figure 5 Effect of DAZM on sex hormones in female rats with SKYD. *, in comparison with N group, P<0.05; #, in comparison with Model group, P<0.05; ##, in comparison with M group, P<0.01. E2, estradiol; T, testosterone; DAZM, decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency.

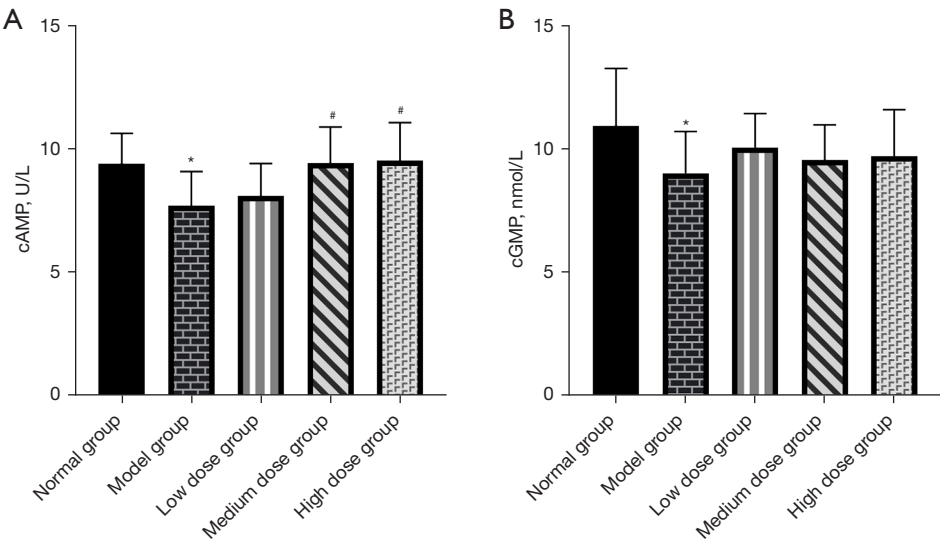

Figure 6 Effect of DAZM on cAMP and cGMP in female rats with SKYD. *, in comparison with N group, P<0.05; #, in comparison with Model group, P<0.05. cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DAZM, decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency.

table that there are no marked changes in vitamin C, BLD, PRO, WBC, and ketone body (ket) of female rats. The PH value of rats in the normal group was markedly higher than that in the 3 dose groups of the DAZM and Model groups (P<0.05), but there was no marked difference between the 3 dose groups of the DAZM and Model groups (P>0.05).

Effect of DAZM on histopathology of female rats with SKYD

The microscopic findings of histology are shown in *Figures 8-10*. There were no abnormalities in tissue structure and cell morphology of rats' kidneys in the N group. In

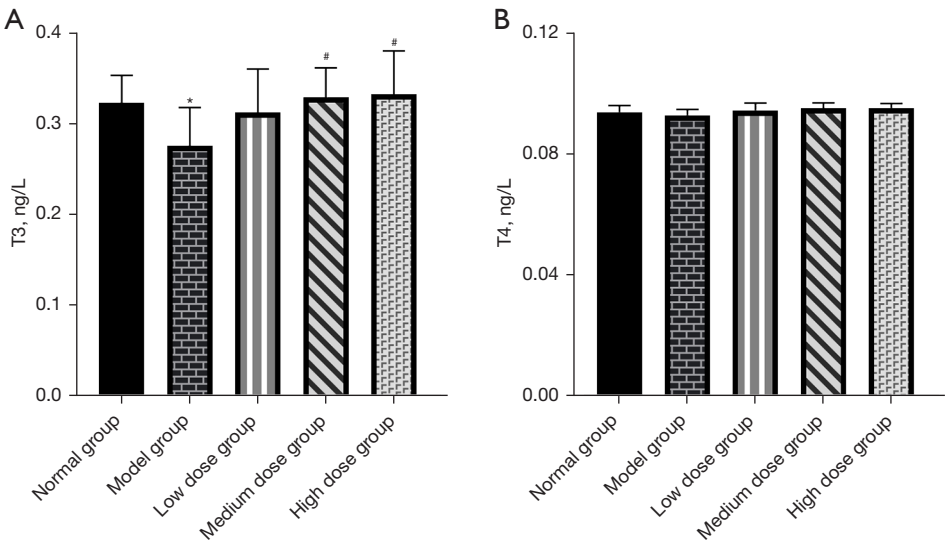

Figure 7 Effect of DAZM on thyroxine in female rats with SKYD. *, in comparison with N group, P<0.05; #, in comparison with Model group, P<0.05. T3, triiodothyronine; T4, thyroxine; DAZM, decoction of *Angelica sinensis*, *Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency.

Table 3 Effect of DAZM on end-stage urine routine of female rats with SKYD

Group	PH	Vitamin			Protein			Occult blood			White blood cell			Ketone body		
		−	+	±	−	+	±	−	+	±	−	+	±	−	+	±
N group	8.2±0.7	4	5	3	4	4	4	9	2	1	8	0	4	9	0	3
Model group	6.2±1.0	4	5	3	8	1	3	10	1	1	8	0	4	12	0	0
L group	7.2±0.6	6	0	6	9	2	1	6	5	1	9	1	2	12	0	0
M group	6.7±0.5	6	1	5	9	3	0	9	2	1	8	0	4	12	0	0
H group	6.9±0.6	4	1	7	12	0	0	7	4	1	9	1	2	12	0	0

DAZM, decoction of *Angelica sinensis, Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency; BW, body weight; N, normal group; Model, model control group; L, low dose group; M, medium-dose group; H, high dose group.

comparison with the N group, the renal tissue of the Model group indicated obvious pathological changes, mainly manifested in the reduction of the number of glomeruli and hyperemia under the same multiple field of view. As shown in *Figure 8*, sacculus synechia, enlargement of capsular cavity, yellowish brown crystals of different sizes and numbers were present in the renal tubular lumen, interstitial fiber tissue hyperplasia and inflammatory cell infiltration, turbidity and swelling of renal tubular epithelial cells, and exudation of some lumen proteins. After the intervention of DAZM, the pathological condition of kidney was improved, and the phenomena of glomerular congestion, interstitial cell proliferation, and renal tubular protein tube type were markedly improved. Atrophy of splenic corpuscles could be seen in the spleen, whereas after feeding on DAZM, it could be seen that the splenic corpuscle tended to be normal. Uterine changes were not obvious in each group.

Evaluation results of BDI-GS

Table 4 shows the BDI-GS evaluation results of the organ weight of female rats. Two BDI-GS evaluation systems were established here: BDI1 is in comparison with N group and BDI2 is in comparison with Model group. It can be seen from *Table 5* that in comparison with the N group, except for the uterus, the BDI values of the main organs in each

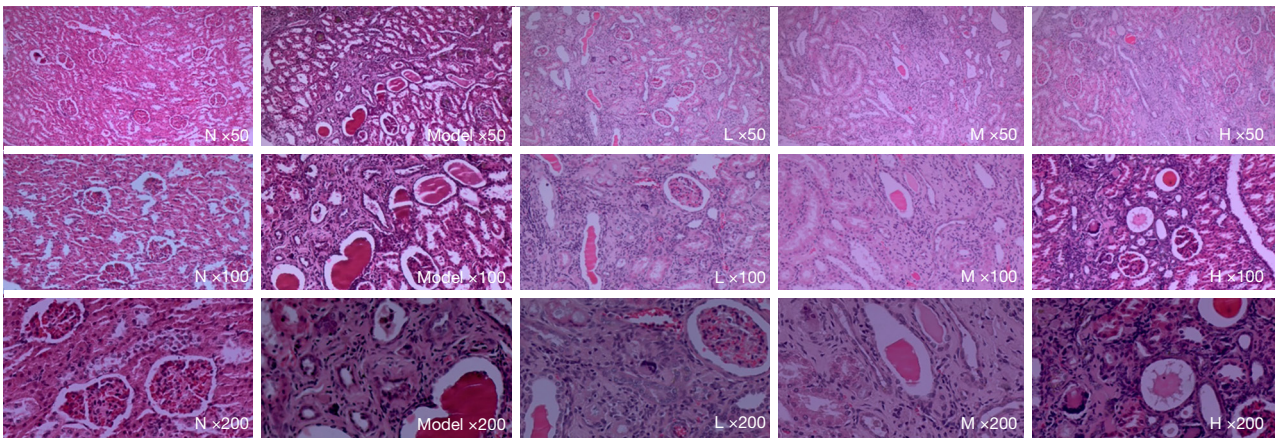

Figure 8 Pathological changes of kidney tissue of rats in each group (HE, ×50, ×100, ×200). N, normal group; Model, model control group; L, low dose group; M, medium-dose group; H, high dose group; HE, hematoxylin and eosin.

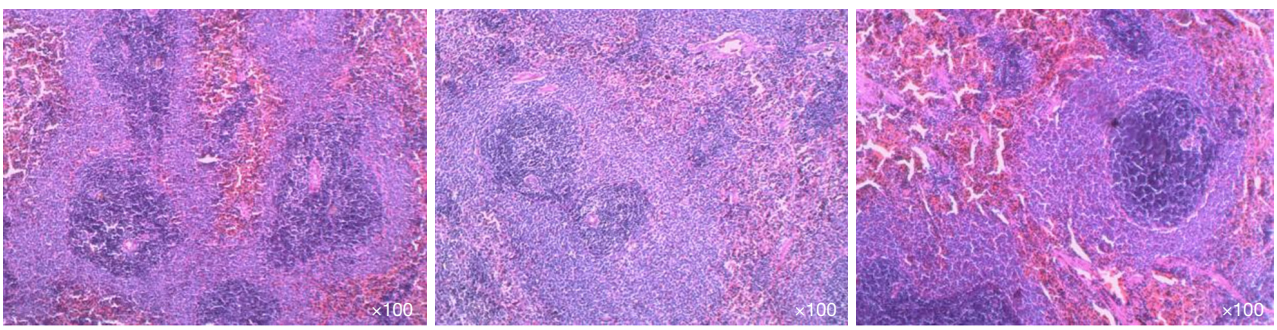

Figure 9 Pathological changes of spleen tissue of rats in each group (HE, ×100). N, normal group; Model, model control group; H, high dose group; HE, hematoxylin and eosin.

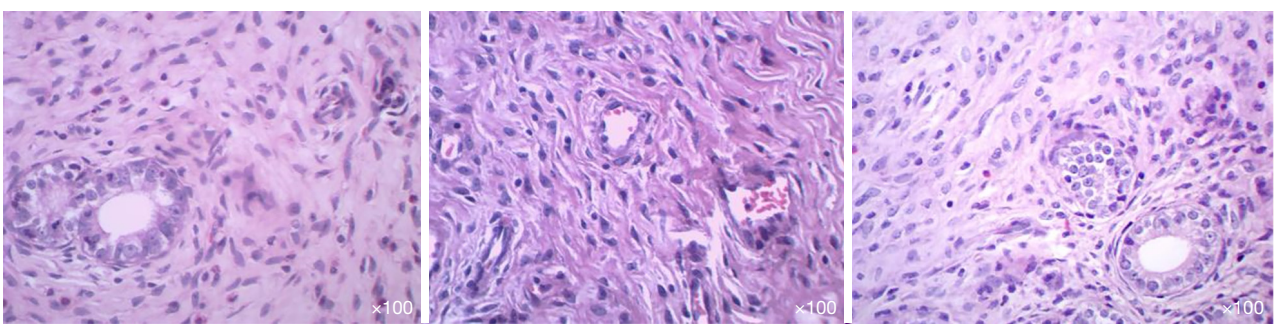

Figure 10 Histopathological changes of uterus tissue of rats in each group (HE, ×200). N, normal group; Model, model control group; H, high dose group; HE, hematoxylin and eosin.

Table 4 BDI-GS evaluation results of organ weight of female rats

Organ	N group	Model group		L group			M group			H group		
		Organ coefficient	BDI1	Organ coefficient	BDI1	BDI2	Organ coefficient	BDI1	BDI2	Organ coefficient	BDI1	BDI2
Heart	0.313	0.337	1.08	0.323	1.03	0.96	0.342	1.09	1.02	0.315	1.01	0.93
Liver	2.663	2.791	1.05	2.899	1.09	1.04	3.221	1.21	1.15	2.887	1.08	1.03
Spleen	0.195	0.233	1.19	0.251	1.29	1.08	0.266	1.36	1.14	0.26	1.33	1.12
Lung	0.423	0.432	1.02	0.464	1.10	1.07	0.492	1.16	1.14	0.466	1.01	1.08
Kidney	0.670	1.280	1.91	1.306	1.94	1.02	1.374	2.05	1.07	1.391	2.07	1.09
Brain	0.458	0.468	1.02	0.476	1.04	1.02	0.500	1.09	1.07	0.505	1.10	1.08
Thymus	0.097	0.103	1.05	0.093	0.96	0.46	0.096	1.00	0.47	0.112	1.15	0.55
Ovary	0.056	0.057	1.02	0.063	1.13	1.11	0.072	1.29	1.26	0.06	1.07	1.05
Uterus	0.256	0.207	0.81	0.214	0.83	1.03	0.195	0.76	0.94	0.223	0.87	1.08
Renal capsule	0.024	0.025	1.04	0.031	1.29	1.24	0.034	1.42	1.36	0.032	1.33	1.28
GS	–	–	11.19	–	11.74	10.03	–	12.43	10.62	–	12.02	10.29

BDI-GS, benefit damage index-general score; N, normal group; Model, model control group; L, low dose group; M, medium-dose group; H, high dose group.

Table 5 Effect of DAZM on visceral body ratio of female rats with SKYD ($\bar{x} \pm S$)

Group	Wet weight of liver (g)	Liver/body weight (%)	Wet weight of spleen (g)	Spleen/body weight (%)	Wet weight of kidney (g)	Kidney/body weight (%)	Wet weight of uterus (g)	Uterus/body weight (%)
N group	7.30±0.69	2.63±0.23	0.56±0.10	0.20±0.04	1.72±0.14	0.62±0.04	0.72±0.20	0.26±0.07
Model group	7.54±0.59	2.79±0.26	0.61±0.06	0.23±0.03	3.34±0.41	1.18±0.17*	0.52±0.26	0.21±0.09
L group	7.91±0.62	2.90±0.20	0.68±0.09	0.25±0.04	3.54±0.47	1.31±0.23*	0.57±0.17	0.20±0.08
M group	8.38±0.75	3.19±0.26	0.71±0.09	0.27±0.03	3.60±0.55	1.37±0.24*	0.51±0.16	0.19±0.06
H group	7.70±0.65	2.89±0.25	0.69±0.14	0.26±0.05	3.70±0.90	1.39±0.36*	0.60±0.22	0.22±0.08

*, in comparison with N group, P<0.05. DAZM, decoction of *Angelica sinensis, Zingiberis Rhizoma Recens*, and mutton; SKYD, spleen-kidney Yang deficiency; BW, body weight; N, normal group; Model, model control group; L, low dose group; M, medium-dose group; H, high dose group.

treatment group are >1, and the GS level is in the range of 11.19–12.43. After feeding DAZM, the BDI values of liver, spleen, lung, kidney, brain, ovary, and adrenal gland of female rats in the 3 dose groups are also >1, and the total cumulative GS values of organs in each group are stable at the level of 10.03–10.62.

Discussion

In this study, female rats with SKYD were fed with DAZM for 30 days to examine the effects of DAZM on the physiological and biochemical indexes of rats in the administration group, and combined with BDI-GS evaluation index system to evaluate the tonifying effect of DAZM.

The weight of the rats in N group grew linearly, but the weight of the rats in the experimental group reduced due to the SKYD caused by the intake of adenine and senna leaf water. After administration, the weight of DAZM in each experimental group grew in comparison with the Model group, but the difference was not marked. After modeling, the water consumption of rats in the experimental group grew. After administration, there was no marked difference between the DAZM dose group and M group, whereas

the food consumption of rats in the high-dose group was markedly lower than that of other groups. The possible reason is that the large proportion of mutton fed reduced the food consumption of rats, and there was no marked difference between other groups. The SKYD led to the marked reduction of anal temperature in rats. After intragastric administration of DAZM, there was no marked difference observed in the elevation of anal temperature between the administration and normal group, suggesting that DAZM can restore the anal temperature to normal.

Hematological examination showed that the WBC and PLT of female rats in the SKYD model group were markedly higher than those in N group ($P<0.05$). There was no marked difference observed in WBC and PLT between the DAZM and N group. The TB and TG of female rats in Model group decreased markedly, and DAZM increased the TB and TG, which was markedly different from that in the Model group, and related to the high fat content of DAZM. Routine urine examination found that the BUN and CRE of the M group and the experimental group were markedly higher than those of N group, and there were marked differences. Combined with the results of routine urine examination, the urine acidification of the SKYD group changed markedly. After taking DAZM, the pH value, protein, and biochemical indexes of urine tended to be normal.

The SKYD caused by intake of adenine and senna water led to the marked reduction of E2 in the model group in comparison with N group ($P<0.05$). After intragastric administration of DAZM, E2 grew markedly, and there was no marked difference in comparison with the N group. It can be deduced that DAZM can restore E2 to normal. The chemical substance known as cAMP is a weighty substance which is involved in the regulation of substance metabolism and biological function in cells. The cyclic nucleotide cGMP functions to transmit intracellular information and then transmit extracellular signals to the nucleus (20). Many researchers have reported on the relationship between cyclic nucleotides and "Yin and Yang" in TCM (21-23). Goldberg put forward the "Yin Yang Theory" of cell function regulation in the early 1970s (22). He realized that cAMP and cGMP are generally antagonistic and participate in the regulation of cellular response. The dualistic theory of biological control of "Yin and Yang" was further proposed. It is believed that this antagonism can be "Yin" and "Yang". The concentration and action of cGMP and cAMP in cells are in opposition to each other. For example, when the level of cAMP in cells growths, glycogen is decomposed into Glu, which promotes the expression of cell genes and the synthesis of specific proteins, so as to make cells differentiate. The growth of cGMP promotes Glu synthesis of glycogen, accelerates DNA replication, cell division, and proliferation, but the effects of cGMP and cAMP on body temperature are opposite (24). The cAMP/cGMP ratio has certain correlation with Yang insufficiency constitution (25). Growth of cAMP content indicates the growth of cell anabolism and body temperature. DAZM can elevate rectal temperature and affect the cAMP/cGMP level.

BDI (benefit damage index): the mean value of the statistical value of the visceral indicators of the test substance/the mean value of the corresponding indicators of the control group, GS (general score), based on the BDI value, is used as the evaluation indicator of the overall comprehensive benefits and health effects through the cumulative score (26-28). In terms of organ coefficient and BDI-GS index, the BDI values of the liver, spleen, lung, kidney, brain, ovary, and adrenal gland of female rats in the 3 administration groups of DAZM were >1, which showed that DAZM facilitated the recovery of organ coefficient of liver, spleen, lung, kidney, brain, ovary, and adrenal gland of female rats, whereas the BDI value of thymus of female rats was <1. It is suggested that adenine and *folium sennae* water can inhibit the function of thymus in female rats, and DAZM has no improvement. However, the total cumulative GS value of each organ of female rats was more than 10, suggesting that the 30 days feeding of DAZM has no harmful effect on the organs of female rats with SKYD, and is conducive to the recovery of animals with SKYD.

Conclusions

Through the modification of physiological and biochemical indexes of experimental animals after 30 days of feeding test, combined with the BDI-GS index system, the results suggest that DAZM has a certain tonifying effect on female rats with SKYD. However, the disadvantage of the paper is that there was no physiological and biochemical detection before medication after modeling, and the physiological changes of rats with SKYD cannot be fully elucidated, which will be improved in future experiments.

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Footnote

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Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6442/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6442/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. JZLLSC20210053) granted by experimental animal Ethics Committee of Jiangxi University of Chinese medicine, in compliance with Jiangxi University of Chinese medicine guidelines for the care and use of animals.

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