Effect of *Boschniakia Rossica* Extract on BMP-5 and ActRIIA in Mice Model of Kidney-Yang Deficient

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Research

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Abstract

Background *Boschniakia rossica* (Cham. & Schltdl.) B. Fedtsch. (Bu-Lao-Cao in Chinese name) is a famous Chinese traditional drug and mainly distributed in Korea, Japan, Russia, and China. The dried whole grass of *Boschniakia rossica* is commonly used in traditional Chinese medicine to treat kidney deficiency, yang deficiency, waist and knee aches and constipation. In order to quantify the active ingredients in *B. rossica* chinensis and body weight, clinical features and serum SOD and MDA activities, cAMP, cGMP, and testosterone (T), follicle-stimulating growth hormone (FSH), mouse bone morphogenetic protein-5 (BMP-5), mouse activin receptor IIA (ActRIIA) were measured to elucidate the mechanism underlying its treatment of kidney yang deficiency potential.

Methods Extracts of *B. rossica* were evaluated by HPLC-MS analyses. Kidney-yang deficiency syndrome was induced in mouse with hydrocortisone (10 mg/Kg, 15d) and treated with *B. rossica* extract. The animals were treated orally with 3 doses of B. rossica extract: 1000, 500 and 250 mg/Kg, for 15 days. Body weight and clinical characteristics were recorded daily. For biochemical analyses, the following tests were performed: superoxide dismutase (SOD) activity, malondialdehyde (MDA) activity, testosterone (T) content, follicle growth hormone (FSH) content, cyclic adenosine monophosphate (cAMP) content, mouse bone morphogenetic protein-5 (BMP-5) content and activin receptor IIA (ActRIIA) content.

Results Our study found that all the mice except the blank group showed significant systemic depletion caused by hydrocortisone. Low T content, low cAMP activity and high cGMP content were consistent with the physiological and biochemical indexes of kidney-yang deficiency syndrome. After oral gavage, the pathological conditions were improved in both the Positive group and the B. rossica groups.

Conclusions The high-dose extract of *B. rossica* showed similar therapeutic effects as Jingui Shenqi Pill. The extract of *B. rossica* can be used to treat with hydrocortisone-induced kidney yang deficiency syndrome by increasing serum testosterone content and decreasing FSH content. More importantly, the experiment found that activin A and BMP-5 also have effects on kidney yang deficiency syndrome.

Background

Dried whole grass of *B rossica* was often used as a supplement or vitality drug to enhance renal function and sexual dysfunction in China [1]. The extract of *B. rossica* has a variety of pharmacological activities, including anti-oxidation, anti-inflammatory, anti-tumor, and improving animal memory [2–3]. This study was mainly to study the therapeutic effect of extract of *B. rossica* on mice with kidney yang deficiency syndrome.

Modern studies have shown that the pathogenesis of KYDS is mainly characterized by different degrees of dysfunction of the hypothalamic-pituitary-target gland axis (adrenal gland, thyroid gland and gonad), which is characterized by diarrhea, cold limbs, decreased mobility, unresponsiveness, loss of appetite, Weight loss, sputum and polyuria[4–6]. Activin has a certain influence on the regulation of gonadal hormone in the hypothalamic-pituitary-target gland axis, and may affect the formation of bone protein,

which providing more scientific explanation for the kidney yang deficiency syndrome. Activin A is a member of the transforming growth factor-beta superfamily, which has endocrine and paracrine effects and that is at the heart of male reproductive biology and health [7].

Bone morphogenetic protein (BMP) is a secreted protein, which belonging to the transforming growth factor-beta (TGF-beta) superfamily. They play an important role in bone formation, heart and cartilage formation and neurodevelopment [8–11].

Materials And Methods

Materials and chemicals

B. rossica was purchased in November 2018 from the local herbal market (Harbin, China). A voucher specimen (100002001002001) was deposited at the herbarium of the Northeast Forestry University for future reference. Hydrocortisone injection was purchased from Tianjin Jinyao Amino Acid Co., Ltd. (Tianjin, China). Jingui Shenqi Pill was purchased from Beijing Tongrentang Technology Development Co., Ltd. (Beijing, China). Mouse follicle stimulating hormone (FSH), testosterone (T), cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), superoxide dismutase (SOD) and malondialdehyde (MDA) colorimetric kits were purchased from Nanjing Established the Institute of Bioengineering (Nanjing, China). Mouse bone morphogenetic protein 5 (BMP-5), mouse activin receptor IIA (ActRIIA), enzyme-linked immunosorbent assay kit was purchased from Jianglai Bio (Shanghai, China).

Preparation of sample solutions and standard solutions

The grass B. rossica was dried and pulverized, passed through a 60-mesh screen to reserve. Accurately weighed 1.00 g dried powder of B. *rossica*, added 50 ml of methanol in an Erlenmeyer flask, ultrasonically extracted for 30 min, and make up the weight with methanol. A 5 ml of the filtrate was evaporated and dried, dissolved in a 10 ml volumetric flask with a chromatographic methanol and diluted to the mark, then filtered through a microporous membrane (0.22 μm), and the solution was stored in a refrigerator at 4 ℃ to reserve.

Chromatographic conditions

A Klimail 100-5 C18 column (250 mm × 4.6 mm, 5 μm) was used for the detection and analysis using an Agilent Model 1260 HPLC. Mobile phase composition: gradient elution with acetonitrile (A) – 0.5% formic acid in water (B). Gradient elution conditions: 0–12 min, 15% (A); 12–30 min, 15–20% (A); 30–40 min, 20–25% (A); 40–45 min, 25–30% (A); 45–60 min, 30–100% (A). The flow rate was 1.0 mL/min, the injection volume was 20 μL, the UV detection wavelength was 260 nm, and the column temperature was 30 ℃.

To identify the components, a liquid chromatography mass spectrometer (Waters Quattro premier XE, USA) was used. MS detection parameters are as follows: electrospray ion source (ESI); detection method

is negative ion detection; scanning range m / z 50 ~ 1400; target relative molecular mass 500; drying gas temperature 350 ℃; dry gas volume flow rate 9.0 L / min; The atomizing gas pressure is 0.24 MPa (35.0 psi); the capillary voltage is 4 KV.

Animal treatment

Eighty male Sprague-Dawley (SD) mice were randomly divided into 8 groups, 10 in each group, weighing approximately 20–26 g. 8 groups included blank control mice [B], 4.5 g/kg positive control mice [C], KYDS model mice [M], 100 mg/kg Acteoside [Acte-H], 50 mg/kg Acteoside [Acte-L], 1000 mg/kg of *B. rossica*-administered mice [BR-H], 500 mg/kg of *B. rossica* - administered mice [BR-M], and 250 mg/kg of *B. rossica* administered mice [BR-L]. Mice were intraperitoneally injected with hydrocortisone 10 mg /kg once a day for 15 consecutive days to induce the KYDS model. Fifteen days later, different doses of *B. rossica* was intragastrically administered to KYDS mice for 15 consecutive days. The mice were euthanized 24 h after the last administration. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Harbin Medical University and approved by the Ethics Committee of the Harbin Medical University [IACUC No. HMUASP(SE)1910117].

Statistical analysis

All experiments were repeated three times. Data were expressed as mean ± SD (standard deviation). All results were analyzed by SPSS (USA) 11.5 statistical analysis software. A P value of less than 0.05 was statistically significant.

Results

The overall process of the experiment is shown in Fig. 1. Figure 2 and Table 1 are the HPLC plots of the grasshopper and the main compounds identified by LC-MS.

Table 1
material of B. *rossica* on LC-MS

Number	Time	Molecular weight	Molecular formula	Compound name
1	15.817	360.36	C16H24O9	8-epi-Deoxyloganic acid
2	16.726	344.45	C16H24O8	Boschnaloside
3	18.204	786.61	C35H46O19	Rossicaside A
4	21.978	161.18	C10H10NO	Boschniakine
5	28.918	798.71	C36H46O20	Rossicaside K
6	30.061	782.75	C36H46O19	Rossicaside I
7	37.671	782.74	C36H46O19	Rossicaside B
8	39.376	620.41	C30H36O14	Rossicaside C

After oral administration of 9 times of B□rossica extracts, the mice developed blinking, unresponsiveness, lethargy, black stools and secrets, and no acute symptoms such as exophthalmos, restlessness, convulsions, shortness of breath, etc. In response, some of the mice lost their luster, but returned to normal within 2–3 days, and no mice died after 7 days of continuous observation. As shown in Table 2, the maximum tolerated dose and the half lethal dose of the mice could not be measured due to the limitation of the administration volume and the concentration of the administration, and it was considered that oral administration of the herbicide was safe.

Table 2
Acute toxicity test.

Medicine	Test concentrations (mg/kg)
B□rossica extracts	10.00, 8.00, 6.40, 5.12, 4.02, 3.28, 2.26, 2.10, 1.68
Control	4.50, 3.60, 2.88, 2.30, 1.84, 1.48, 1.18, 0.94, 0.75

Jingui Shenqi Pill is the highest dose of 18 times the dosage of more than 60 kg of adults, and the lowest dose is 4.5 times. Continuous observation for 7 days without acute toxic reactions such as restlessness, convulsions, dyspnea, and exophthalmos, no mice died. Therefore, oral administration of Jingui Shenqi Pill is considered safe.

There was no difference in body weight between the groups before modeling ($P > 0.05$).

As shown in Fig. 3A, after 15 days of intraperitoneal injection of large dose of hydrocortisone, the body weight of the other groups was significantly lighter than the blank group ($P > 0.05$). As shown in Fig. 3B, after 15 days of administration the positive group, the BBR-L group and Acte-H group were heavier than the model group. As can be seen from Fig. 3C, the weight change and the rate of weight change in the other groups were significantly slower than that in the blank group ($P > 0.05$). As shown in Fig. 3D, the weight and weight change of BBR-L and Acte-H mice before and after treatment were significantly higher than that of the model group ($P > 0.05$). The results showed that the BBR group and the Acte-H group had significant differences in improving the body weight of the mice.

As shown in Fig. 4A, the SOD activity in the serum of the BBR-H,-M groups and the Acte-H group was significantly increased compared with the model group, and the difference was statistically significant ($P < 0.05$). Compared with the blank group, serum SOD activity was statistically significant in the BBR-M group and Acte-H group, and the difference was statistically significant ($P < 0.05$).

As shown in Fig. 4B, compared with the model group, the content of MDA in the serum of the BBR group and the Acte group decreased, and the difference was statistically significant ($P < 0.05$). There was no significant difference in MDA between the BBR group and the Acte group compared with the blank group.

The results showed that the model group mice exhibited KYDS status after treatment, while the positive group, BBR group and Acte group improved hydrocortisone-induced KYDS.

As shown in Fig. 5A, the T content in the serum of the BBR groups and the Acte groups were significantly increased as compared with the model group, and the difference was statistically significant. ($P < 0.05$) Compared with the blank control group, the serum T activity of the BBR-M group and the Acte-H group was significantly increased, and the difference was statistically significant.

As shown in Fig. 5B, the FSH content in the serum of the BBR group and the Acte group was significantly decreased as compared with the model group, and the difference was statistically significant. ($P < 0.05$) Compared with the blank control group, the serum FSH activity of the BBR-M, L groups and the Acte-L group decreased significantly, and the difference was statistically significant.

As shown in Fig. 5C, the cAMP activity level in the serum of the BBR groups and the Acte groups were significantly increased as compared with the model group, and the difference was statistically significant. ($P < 0.05$) Compared with the blank control group, serum cAMP activity was significantly increased in the BBR-H group and the Acte-H group, and the difference was statistically significant.

As shown in Fig. 5D, the content of cGMP in BBR group and the Acte group was significantly lower than that in the model group, and the difference was statistically significant ($P < 0.05$). Compared with the blank control group, the serum cGMP level of the BBR-H group and Acte-H group decreased significantly, and the difference was statistically significant. There was no significant change in serum cGMP content in the BBR-M, -L and the Acte-L groups compared with the blank group.

The results showed that the mice in the model group showed KYDS status after treatment, while the positive group, BBR group and Acte group significantly improved T, FSH, cAMP and cGMP in the serum of KYDS mice.

As shown in Fig. 6A, the ActR2A content in the serum of the BBR group and the Acte group was significantly increased as compared with the model group, and the difference was statistically significant ($P < 0.05$). The activity of serum ActR2A in the BBR-H group and the Positive group was significantly higher than that in the blank control group, and the difference was statistically significant.

As shown in Fig. 6B, serum BMP-5 levels in the BBR-M, -L Acte-H and -L groups were significantly higher than those in the model group, and the difference was statistically significant ($P < 0.05$). Compared with the blank control group, the serum BMP-5 activity of the Acte-H group was significantly increased, and the difference was statistically significant.

The results showed that the mice in the model group showed KYDS status after treatment, while the positive group, BBR group and Acte group significantly improved the serum levels of ActR2A and BMP-5 in KYDS mice.

Discussion

KYDS is one of the basic syndromes of traditional Chinese medicine. It is characterized by weakness, fatigue, waist and knee pain, easy to suffer from loose bones, memory loss, and decreased sexual

function.[4,5] *B. rossica* is a traditional Chinese medicine, attending kidney deficiency and impotence, constipation, and also has a certain effect on rheumatoid arthritis. Therefore, we focus on oxidative stress, hormone immunity, energy metabolism and bone metabolism to study the possible mechanism of BBR extract on KYDS.

Oxidative stress, which is defined as the cell's antioxidant defense system, produces an imbalance between reactive oxygen species (ROS) and is related to the high content of polyunsaturated fatty acids in the sperm plasma membrane due to the decline in fertility. Removal of Seminal plasma during semen storage may reduce the ability to protect enzymes and make sperm more susceptible to oxidative stress [12–13]. Superoxide dismutase (SOD) plays a fundamental but indispensable role in the antioxidant protection of biological systems against free radical attack. The enzyme is essential for cell health and protects the body's cells from excess oxygen free radicals, free radicals and other harmful substances that promote aging or cell death [14]. Malondialdehyde (MDA) is a reactive end product of lipid peroxidation. Lipid peroxidation products, which readily form adducts with nucleophilic groups of most cells, including DNA, lipids, peptides and proteins, leading to cellular metabolic disorders [15]. SOD and MDA are in a dynamic equilibrium state under physiological conditions, so as to control the content of reactive oxygen species and maintain cell function [16]. Recent studies have shown that low testosterone levels in diabetic men are associated with impaired metabolic status and mitochondrial function, as well as increased inflammation and leukocyte-endothelial cell interactions [17].

cAMP and cGMP are second messengers required for long-term enhancement (LTP) and memory formation/consolidation [18]. Changes in cyclic nucleotide signaling may lead to neurodegenerative diseases Alzheimer's disease, Huntington's disease and Parkinson's disease [19]. Studies have shown that cGMP and cAMP signaling pathways play an active role in osteoblast proliferation and differentiation. It has been observed that elevated intracellular cAMP enhances BMP action and increases ALP activity of osteoblasts in experimental animals [20].

The content of testosterone in the organism is closely related to the Nrf2 signal pathway [21]. If the SOD activity decreases and the MDA content increases, the mice will enter the oxidative stress state, which will cause defects in testosterone synthesis [22]. And cAMP can promote synthetic secondary hormones such as adrenocorticotropic hormone in the pituitary. After adrenocorticotropic hormone binds to adrenal cortical cells, it activates AC, increases cAMP content, thus activates protein kinase (PKA), and then activates synthetase such as corticosteroids in the adrenal gland after phosphorylation, thereby increasing testosterone content [23].

Testosterone (T) and FSH are the major endocrine regulators of spermatogenesis, which occurs in the specific microenvironment of the testes. FSH-R is a G-protein coupled receptor (GPCR) bound to FSH. After activation, membrane-bound adenylate cyclase (AC) catalyzed the production of intracellular g-proteins (e.g., stimulated gα), followed by an mediated pathway that stimulated multiple signal cascades. For example, PKA, PKC, PI3K, Akt / PKB and ERK1 / ERK2, to induce stem cell factor (SCF), glial-derived neurotrophic factor (GDNF) and androgen transcriptional binding protein (ABP) and transduction (TF) et

al. The synergy between FSH and T to maximize the spermatogenic output process, which plays a key role in the spermatogenesis process and male fertility [24]. In adult fertile men and women, FSH mediates spermatogenesis and follicular development through its G-protein coupled receptor (FSHR) [25]. Activation of the FSH-induced INHA promoter cyclic AMP (cAMP) response element can be partially enhanced by androgens [4].

Activin is a growth factor in the transforming growth factor beta (TGFβ) superfamily. They have both endocrine and paracrine effects, which are essential for male reproductive biology and health. Areas of activin dysfunction may be linked to male reproductive symptoms, including infertility, inflammation and testicular cancer [7]. TGF-β1 can promote the proliferation and differentiation of osteoblasts, induce osteoclast apoptosis and improve bone formation[26]. Moreover TGF-β1 and activin A block the induction of Srebp1c (an inducer of adipogenesis) and TGF-β1 and activin A can act as inhibitors of adipogenesis [27]. Bone morphogenetic protein (BMP) is a member of the TGF-β family. It plays an important role in biological processes, including the involvement of vascular precursor cells in angiogenesis and the regulation of vascular cell population growth, differentiation and renewal [28]. ATP stimulates the formation of ALP and the expression of bone morphogenetic protein (BMP)-2, -4 and − 5 genes [29]. The action mechanism of the hypothalamic-pituitary-target gland axis is finally formed (Fig. 7).

Our results showed that the serum level of cAMP in the high-dose BBR extraction group (BBR-H) was significantly increased, which improved the balance between cAMP and cGMP, and thus activated the hypothalamic-pituitary-target gland. The axis signaling pathway demonstrates that BBR had a good therapeutic effect on KYDS mice.

Conclusions

Oral BBR-H dose greater than 1000 mg/kg/d in mice with kidney yang deficiency could increase serum testosterone and cAMP levels, and decrease serum FSH and cGMP levels. BBR anti-kidney yang deficiency is similar to Jingui Shenqi Pill. Acteoside was a representative component of phenylpropanol glycosides, phenylpropanol glycosides was the main component of the extract of *B. rossica*. Acteoside seems to be the active ingredient in *B. rossica* which has the ability to regulate ActR2A and BMP-5, but its mechanism of action on KYDS requires further research.

Abbreviations

ABP: Androgen transcriptional binding protein

AC: Adenylate cyclase

ActR□A: Activin □ A receptor

ARE: Antioxidant response element

ATP: Adenosine triphosphate

BMP: Bone morphogenetic protein

BMP-5: Bone morphogenetic protein 5

cAMP: Cyclic adenosine monophosphate

cGMP: Cyclic guanosine monophosphate

FSH: Follicle-stimulating hormone

FSHR: Follicle-stimulating hormone receptor

HPLC: High Performance Liquid Chromatography

KYDS: Kidney-yang deficiency syndrome

MDA: Malondialdehyde

Nrf2: NF-E2-related nuclear factor 2

PKA: Protein kinase A

SCF: Stem cell factor

SOD: Superoxide dismutase

T: Testosterone

Declarations

Availability of data and materials

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

Author Contribution

Conceptualization, M.L. and F.S.M.; methodology, C.M., M.F.T., C.Y.F. and X.M.M.; investigation, C.M., M.F.T., C.Y.F. and X.M.M.; resources, M.L.; writing—original draft preparation, C.M. and M.F.T.; writing—review and editing, C.M. and M.L.; visualization, C.Y.F. and X.M.M.; supervision, M.L. and C.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors have declared that there is no conflict of interest.

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Figures

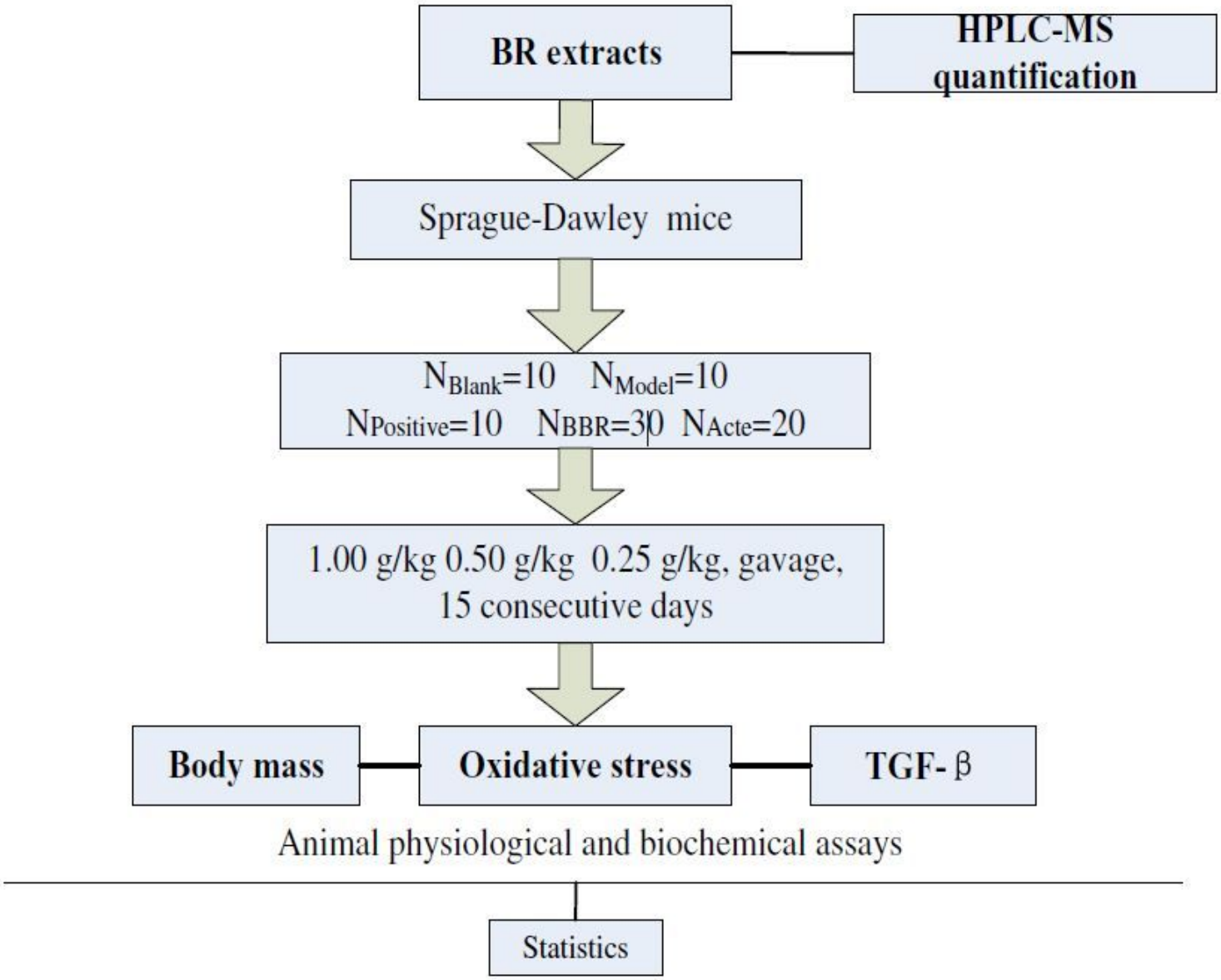

Figure 1

Describes the experimental design.

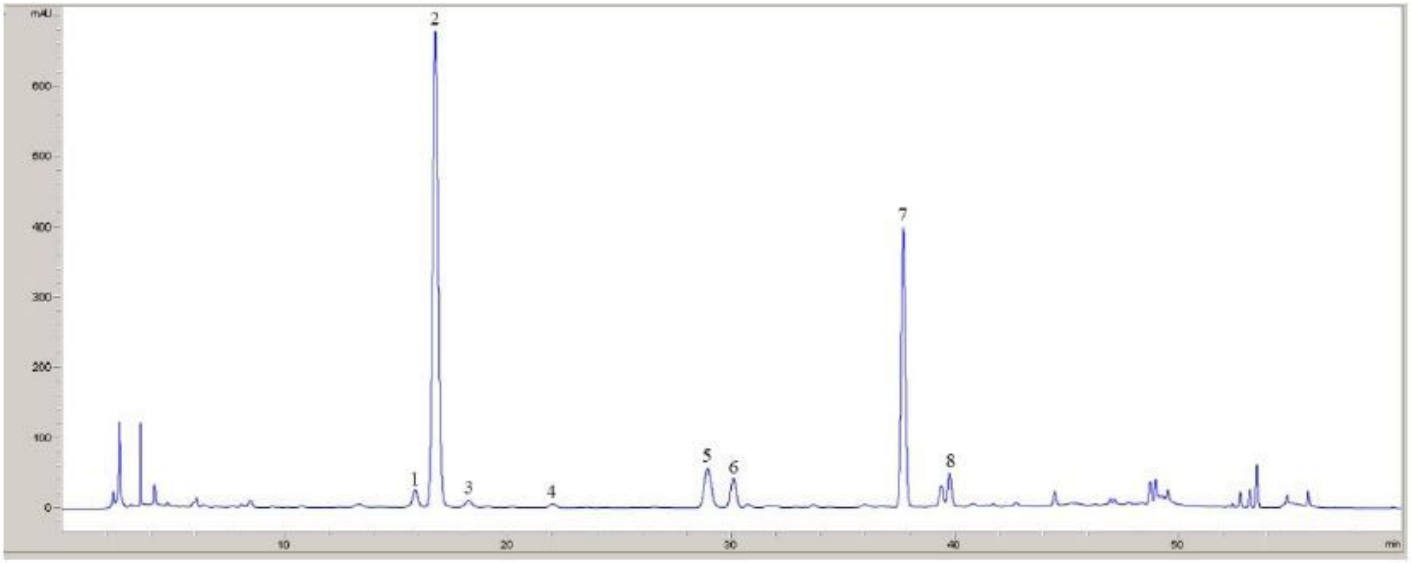

Figure 2

The HPLC of B. rossica

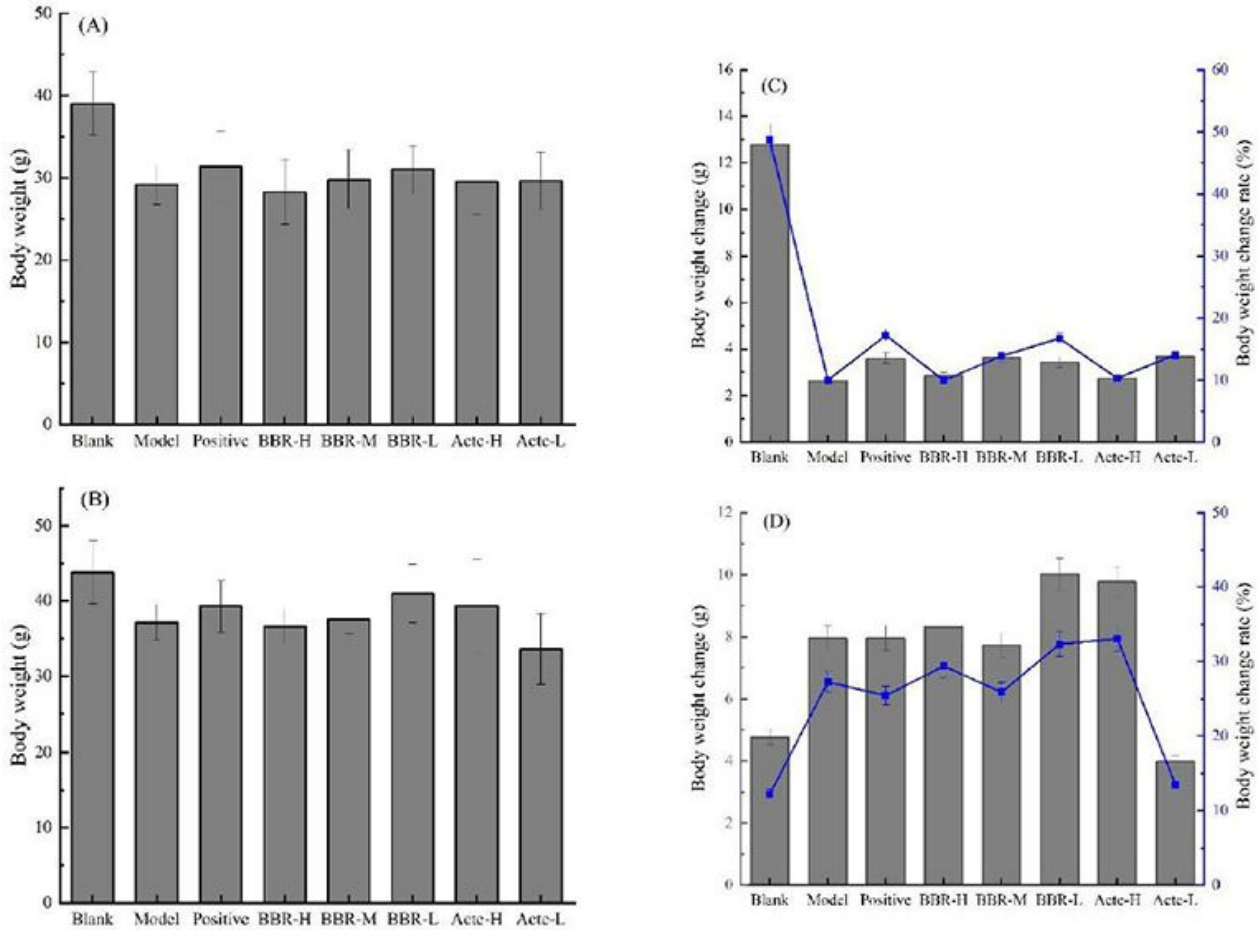

Figure 3

Effect of B. rossica extract on the body weight in mice. (A) is the body mass of the mice after modeling, (B) is the body mass of the mice after administration, (C) is the body mass change value and body mass change rate of the mice before and after modeling, and (D) is the body mass change value and body mass change rate of the mice before and after oral administration.

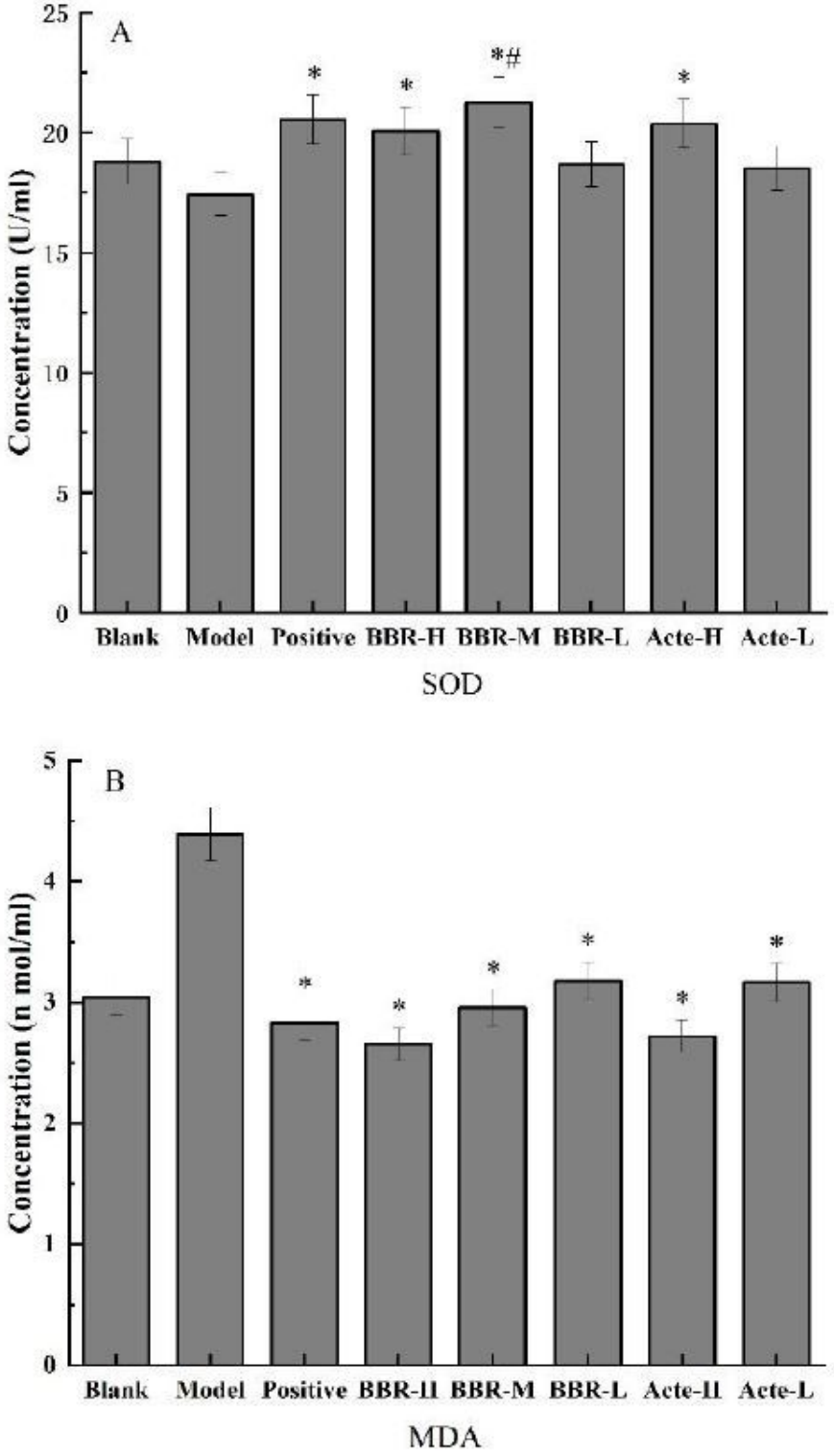

Figure 4

Effect of B. rossica extract on the activity of SOD and MDA. The results are shown in mean ± SEM. * P<0.05, ** P<0.01 compared with the Model group; # P<0.05 compared with the Blank group

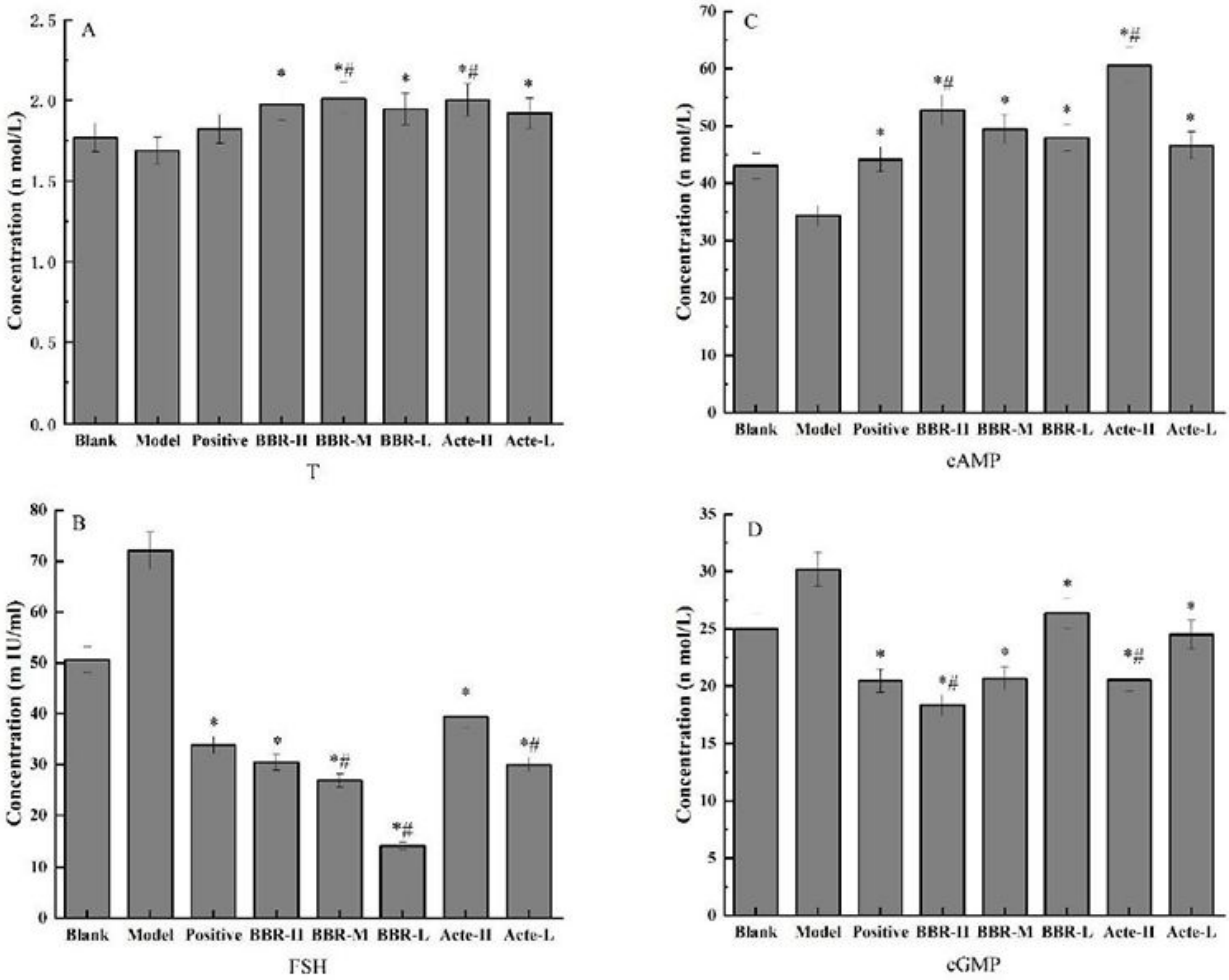

Figure 5

Effect of B. rossica extract on the concentrations of T, FSH, cAMP and cGMP. The results are shown in mean ± SEM. * P<0.05, ** P<0.01 compared with the Model group; # P<0.05 compared with the Blank group.

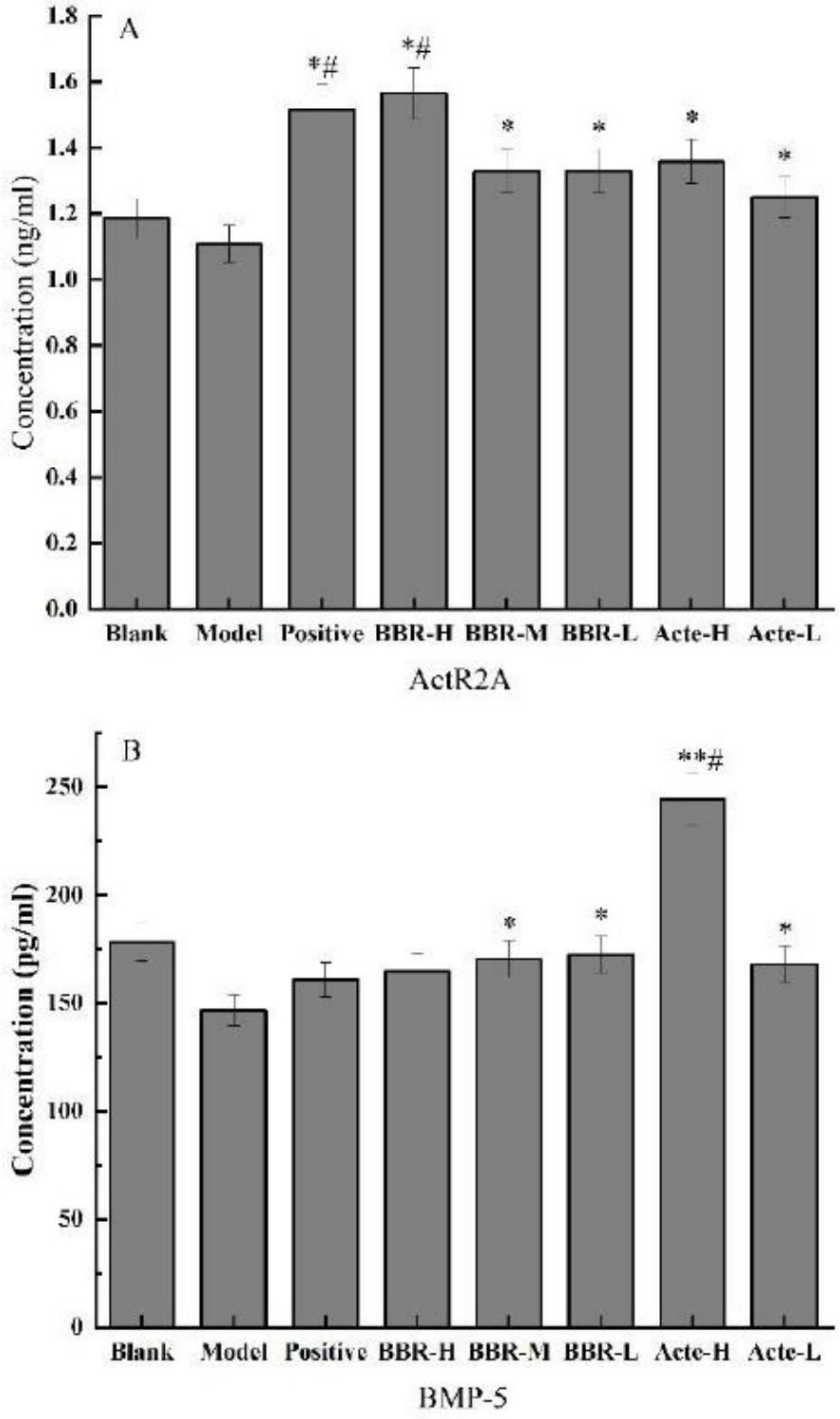

Figure 6

Effect of B. rossica extract on the concentrations of ActR2A and BMP-5. The results are shown in mean ± SEM. * P<0.05, ** P<0.01 compared with the Model group; ° P<0.05 compared with the Blank group.

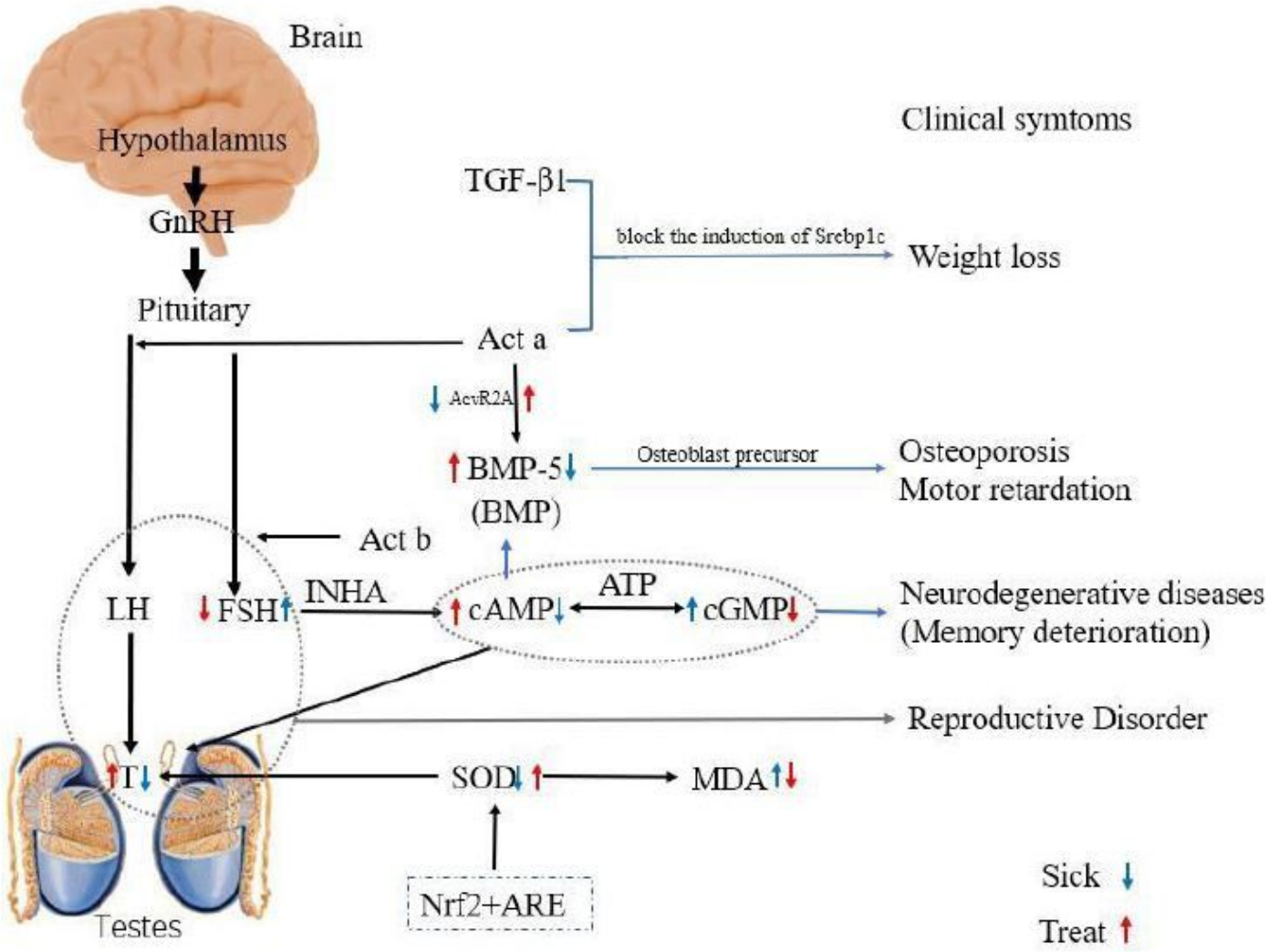

Figure 7

A treatment pathway of the B. rossica extract associated with Kidney-Yang deficiency.