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Effects of Shenxian Congee on Chronic Fatigue Syndrome Rats by NF-κB Signaling Pathway

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ABSTRACT

The purpose of this study was to study the effect of Shenxian congee (SXC) in rats with chronic fatigue syndrome (CFS) on NF-κB signalling pathway and the expression of related factors. To reveal the relevant signal transduction mechanism in the treatment of CFS. Seventy two male SD rats were randomly divided into 6 groups with 12 rats in each group. Namely: control group (CON), low dose Shenxian Congee group (SXC-L), medium dose Shenxian Congee group (SXC-M), high dose Shenxian Congee group (SXC-M), fluoxetine hydrochloride group (influenza group, FLU) and chronic fatigue syndrome (CFS) group. In addition to the CON group, the remaining five groups established CFS rat models through forced swimming test and chronic restraint stress. After 28 days, the three groups received different concentrations of SXC (1.62 g/ml, 0.81 g/ml and 0.41 g/ml), FLU group received 0.21 mg/ml fluoxetine. CFS group and CON group received the same volume of normal saline. All groups received treatment for 28 days. The rats' body weight, fatigue time, activity and mobility, and mRNA and protein levels of interleukin-1 beta (IL-1β), tumour necrosis factor alpha (TNF-α), transforming growth factor β-activated kinase 1 (TAK1), TAB, IκB kinase (IKKα), IκBα, NF-κBp65 and cyclooxygenase-2 in the serum were measured. Compared with the CON group, the CFS group had decreased weight and decreased activity and mobility ($P < 0.05$). Compared with the CFS group, the three SXC groups and the FLU group all had varying levels of increased body weight, horizontal motion, vertical motion and fatigue time ($P < 0.05$). The SXC downregulated the mRNA and/or protein expression of IL-1β, TNF-α, TAK1, TAB, IKKα and NF-κBp65 and increased the mRNA expression of IκBα protein ($P < 0.05$). To conclude in the CFS model, SXC inhibits the transmission of NF-κB signaling pathway by down-regulating the expression of inflammatory cytokines and protein targets, and has an immunoregulatory effect on chronic fatigue syndrome.

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JG and CH designed the study. LH, YZ, SC and XB conducted the experiments and acquired the data. LH, DB and CW analysed the data and wrote the first draft of the manuscript.

Key words
Dietary supplements, Shenxian congee, Chronic fatigue syndrome, Deficiency of spleen and kidney yang, NF-κB signalling pathway

INTRODUCTION

Chronic fatigue syndrome (CFS) has long been a common and frequently occurring clinical disease. It persistent or recurs debilitating fatigue at least six months and causes disturbed sleep patterns, muscle and joint pain, and headaches. This disease can severely impair patients' health and well-being (Sharpe and Greco, 2019; Rollnik, 2017; Chan *et al*., 2014). At present, the main therapeutic methods for CFS are drugs, exercise and psychological treatment. However, the drugs can cause side effects, such as central nervous system dysfunction and an increased risk of fatigue. Exercise therapy needs long-term persistence and tends to aggravate fatigue, leading to poor patient compliance (Larun *et al*., 2017). Also, psychological treatment is often limited in clinical settings due to its high price and personalized needs (Wiborg *et al*., 2012). It is necessary to find therapy with a good curative effect, few side effects and a relatively low price. Recently, research has shown that certain kinds of traditional Chinese herbal drugs could enhance the immune system, maintain homeostasis and postpone fatigue.

Previous studies have found that the occurrence of CFS is correlated with autoimmunity or dysregulated inflammation (Ye, 2017), including natural killer (NK) cell dysfunction (Cliff *et al*., 2019) and pro-inflammatory cytokine activation, e.g., interleukin-1 beta (IL-1β) and tumour necrosis factor alpha (TNF-α) (Morris *et al*., 2018; Milrad *et al*., 2017). According to literature research combined with clinical studies, it is speculated that the occurrence of CFS may be closely related to abnormal

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changes in the inflammation reaction (Missailidis *et al*., 2019; Groven *et al*., 2018). NF-κB is one of main signalling pathways for the inflammation reaction and plays a crucial role in changing the inflammation reaction.

In recent years, reports on the treatment of CFS by traditional Chinese medicine have gradually increased and achieved good curative effects (Lin *et al*., 2017; Cheng *et al*., 2013; Liu and Lei, 2012). Traditional Chinese medicine believes that CFS is related to emotional disorder, eating disorder, overwork, congenital deficiency or exogenous evil. The pathogenesis is mainly the imbalance of five internal organs and the deficiency of Qi and blood. Among them, abnormal spleen and kidney function is the key pathogenesis (Zhao and Jiang, 2014; Zeng *et al*., 2013).

Shenxian congee (SXC) is an effective dietary supplement of traditional Chinese medicine in China. It can be traced back to the ancient book Dunhuang scroll. It is composed of Chinese yam, gordon euryale seed, tuber onion seed and rice fruit (Zhong *et al*., 2018). It has the effects of invigorating spleen and nourishing stomach, warming kidney and consolidating essence, improving immunity and promoting metabolism. According to our previous research results, SXC has a unique effect in the treatment of CFS, which can effectively reduce fatigue, improve the TCM syndrome of patients with chronic CFS, and has small side effects (Wirth and Scheibenbogen, 2020). Its regulatory effect may be related to NF-κB signaling pathway. However, the mechanism of SXC alleviating fatigue has not been studied.

Therefore, we conducted this study to investigate the effect of SXC on the expression of NF-κB signalling pathway–related factors in rats with CFS in order to reveal the relevant signal transduction mechanism by which SXC acts to treat CFS.

MATERIALS AND METHODS

Ethics statement

This study was conducted in strict accordance with the recommendations of the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China. The protocol and experimental designs were approved by the Ethical Committee of Chengdu University of Traditional Chinese Medicine. All possible steps were taken to avoid the animals' suffering at any stage of the experiment. At the end of the study, the animals were sacrificed after being anaesthetised with pentobarbital sodium (100 mg·kg-1).

Experimental animal

SD rats (SPF) weighing 200 ± 20 g were acquired from Chengdu Dashuo Laboratory Animal Technology Co., Ltd (Permit No. SCXK (Chuan) 2015-030, Chengdu, China). The animals were maintained under controlled conditions at a temperature of 20 ± 0.5°C, a humidity of 55 ± 5%, and 12-h light and 12-h dark cycles. Before experiments, the animals were fasted for 24 h with free access to food and tap water.

SXC preparation

Firstly, 10g rice fruit and 100g tuber onion seed were put into their respective food packaging. Adding 800mL water, boiling at high temperature. Then soft fire was boiled for about 25 min, remove the bag. Then, 10 g Chinese yam powder and 10 g gordon euryale seed powder were soaked in the required water and poured into the pot. Mix well and boil for 3 ~ 5 min. Each time add congee about 300 ~ 400 mL. Finally, the filtrate was diluted with distilled water to three doses: high (1.62 g / m L), medium (0.81 g / m L) and low (0.41 g / m L). SXC is fried every two days and stored at low temperature until 37 °C. All materials were provided by the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine. All samples were deposited at Chengdu University of Traditional Chinese Medicine, Chengdu, 611137, China.

Dosage of SXC

The dose conversion coefficient table of animals and normal adults per kg body weight shows that the dose conversion coefficient of rats and normal adults per kg body weight is 6.25. According to the ordinary adult (60kg) daily intake of a dose (containing 80g) cooked food porridge calculation. Adults daily intake about 80g / 60kg = 1.33g/ kg medicated diet. According to the conversion coefficient, the daily dose of rats was 1.33 g/ kg × 6.25 ≈ 8.31 g/ kg. Low, medium and high dose groups were about 4.16g/ kg, 8.31g/ kg, 16.62g/ kg (low, medium and high dose were 1/ 2 times, 1 time, 2 times of daily dose). According to the daily intragastric administration of 1ml/ 100g, the drug concentration of low, medium and high dose groups were 0.42g/ml, 0.83g/ml and 1.66g/ml, respectively.

Rat model induction and experimental protocol

In total, 72 rats were randomly divided into six different groups (n=12), namely the control group (CON), SXC low-dose group (SXC-L), SXC middle-dose group (SXC-M), SXC high-dose group (SXC-H), fluoxetine group (FLU, positive control, 0.21 mg/mL) and CFS group. Fluoxetine is a widely used selective 5-HT reuptake inhibitor that can selectively inhibit 5-HT transporter, block 5-HT reuptake by presynaptic membrane, prolong and increase 5-HT action, and produce an antidepressant effect. It is used clinically for CFS treatment.

The CFS rat model was induced by a forced swimming test (FST) and chronic restraint stress (CRS), single-date FST and double-date CRS for two weeks in all groups, except the CON group. The specific methods were as follows:

The FST was carried out from 8 a.m. The rats were forced to swim individually in a vertical glass jar (100 cm × 50 cm × 50 cm) containing 40 ± 1 cm of water at 25 ± 1°C. This depth was sufficient to ensure that the rats could not escape or touch the bottom of the container. One rat at a time was put in the jar. A rat was considered to be fatigued when its head sank into the water for 10 s (Deng *et al*., 2019). Then, the rat was scooped up immediately to avoid drowning or death, dried with a paper towel and placed back in its home cage. The CRS was carried out from 10 a.m. The rats were randomly restrained in a bottle (20 cm long and 5 cm in diameter) for 2~4 h. The bottle was soft in case of body impairment. During the CRS cycle, the rats were exposed to food and water deprivation. After the CRS intervention, all rats were placed back in their cages, and they had free access to food and water.

The CON group was treated with normal saline (10 mL/kg). All the groups were given by gavage once a day for four weeks. Their body weight was measured weekly from the start to end of the experiment, and the amount of saline/gavage intake was adjusted accordingly. Their urine/stool, fur colour, mental state, activity and squint were recorded every day.

Open-field test (OFT)

The OFT was conducted on 1st~8th weeks using an open field (100 cm × 100 cm × 50 cm) with 25 grids. One rat at a time was placed in the middle grid; the number of times that the rat crossed through the adjacent grids and stood on its hind legs within six minutes was recorded.

Forced swim test (FST)

The FST was conducted as previously reported. The fatigue time (i.e., time in the water) was noted on the 1st~8th weeks.

Tail suspension test (TST)

The procedure followed in this study was previously described by Steru (Steru *et al*., 1985). Briefly, the rats were suspended on a lever (50 cm above the floor); the movements of the animal were recorded on the 1st~8th weeks. The total duration of the test (six minutes) could be divided into periods of agitation and immobility. Absence of any limb or body movement was considered immobility.

Enzyme-linked immunosorbent assay (ELISA)

Serum IL-1β and TNF-α levels were measured by ELISA. The test procedure is strictly in accordance with the kit instructions. The details are as follows: (1) Rewarming: Rewarm all reagents to room temperature. (2) Sample addition: Blank control hole without sample, standard sample hole added standard 50μL, sample hole added sample 50μL. (3) Warming: cover the sealing film, gently shake and mix, and then warm at 37 °C for 60 min. (4) Liquid blending: the concentrated detergent is diluted 20 times with distilled water for standby. (5) Washing: remove the sealing film, discard the liquid, dry, each hole filled with detergent, stand for 1 min after discard. So, repeat five times after drying. (6) Coloration: 50 μL of substrate solution A was added to each well, and then 50 μL of substrate solution B was added. The mixture was gently shaken and mixed, and the color was developed in dark at 37 °C for 15 min. (7) Termination: 50 μL of termination solution is added per well to terminate the reaction. (8) Determination: The absorbance of each hole was measured in order with the wavelength of 450 nm after zeroing with the blank hole.

Real-time polymerase chain reaction (RT-PCR)

The total RNA in the leukocytes was extracted using the Animal Total RNA Isolation Kit reagent and reverse transcribed to cDNA using the Prime Script RT Reagent Kit according to the manufacturer's instructions. Then, RT-PCR was performed on the cDNA samples using the SYBR Premix Ex Tap II Kit. The reaction conditions were as follows: 95°C for 30 s, followed by 45 cycles of denaturation at 95°C for 5 s and extension at 72°C for 30 s. All amplifications were done in triplicate. The data were analysed by the Thermo Scientific PikoReal System. The relative expression of each target gene was analysed by the 2−ΔΔCT method: ΔΔCT = (CT target gene – CT internal control) intervention − (CT target gene – CT internal reference) blank. The information of RT-PCR primers is shown in Table I.

Table I. Primer information for real-time polymerase chain reaction

Gene name	Sequence (5'to3')
TAK1	P CAGAGCAACTCAGCCACCAGCACAG
	PP TGTAGTCGCCACGATCCTCGCTTCT
IKKα	P CCTGGAACAGCGTGCCATTGATCTCT
	PP GCTCCTTGAGAACTCGGTCCTGACTC
IκBα	P ACAACAGTCTGAACTCGCCACCCAAC
	PP GTCGTCCACCAACCGCTCCTTCTTG
COX2	P GGCTTACAAGACGCCACATCACCTAT
	PP TCGTAGGGAGGGAAGGGCAATTAGAA

P, primer sequence; PP, Post-primer sequence.

Statistical analysis

The IBM® SPSS® Statistics 23.0 software was used for the statistical analysis. The continuous variables of normal distribution were expressed as the mean ± standard deviation, the continuous variables of non-normal distribution were expressed as the median (interquartile range), and the categorical variables were expressed as the frequency (percentage [%]). For multiple comparisons, each value was compared by one-way ANOVA following the Dunnett test when each datum conformed to the normal distribution; meanwhile, the non-normally distributed continuous data were compared using non-parametric tests. The counting data were tested by the chi-square test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Body weight of rats

The rats in the CON group had the following characteristics: normal daily food and water intake, normal excretion of urine and stool, smooth fur, good mental state and swift reaction. After the model establishment, the rats in the CFS, FLU, SXC-L, SXC-M and SXC-H groups had the following characteristics: anorexia and oliguria, loose stool, rough and dull fur, mental fatigue, slow reaction, arched back and weight loss. After gavage with SXC, the rats in the SXC-L, SXC-M and SXC-H groups showed significant weight loss compared with those in the CFS group. The weight comparison of the rats in the different groups is shown in Figure 1A and Table II.

Sports activities of rats

The OFT was used to observe the changes in the mental fatigue of the rats, as shown in Table II and Figures 1B and C. The results indicated that SXC could significantly increase the rats' horizontal and vertical motion after the intervention. On Day 7, the rats in the FLU group and SXC-H group showed more horizontal motion ($P < 0.05$) compared with the rats in the CFS group but showed no difference in their vertical motion ($P > 0.05$). On Days 21 and 28, the horizontal and vertical motion significantly increased compared with the CFS group ($P < 0.01$), but there was still a gap compared with the CON group.

Fatigue time of rats

The fatigue time is shown in Table II and Figure 1D. On days 21 and 28, the fatigue time in all groups treated with SXC was shorter than that in the CON group ($P < 0.01$), whereas compared with the CFS group, the FLU, SXC-L, SXC-M and SXC-H groups had longer fatigue times ($P < 0.01$). The SXC-M group and SXC-H group had a shorter fatigue time than the FLU group; however, the SXC-L group did not show that advantage.

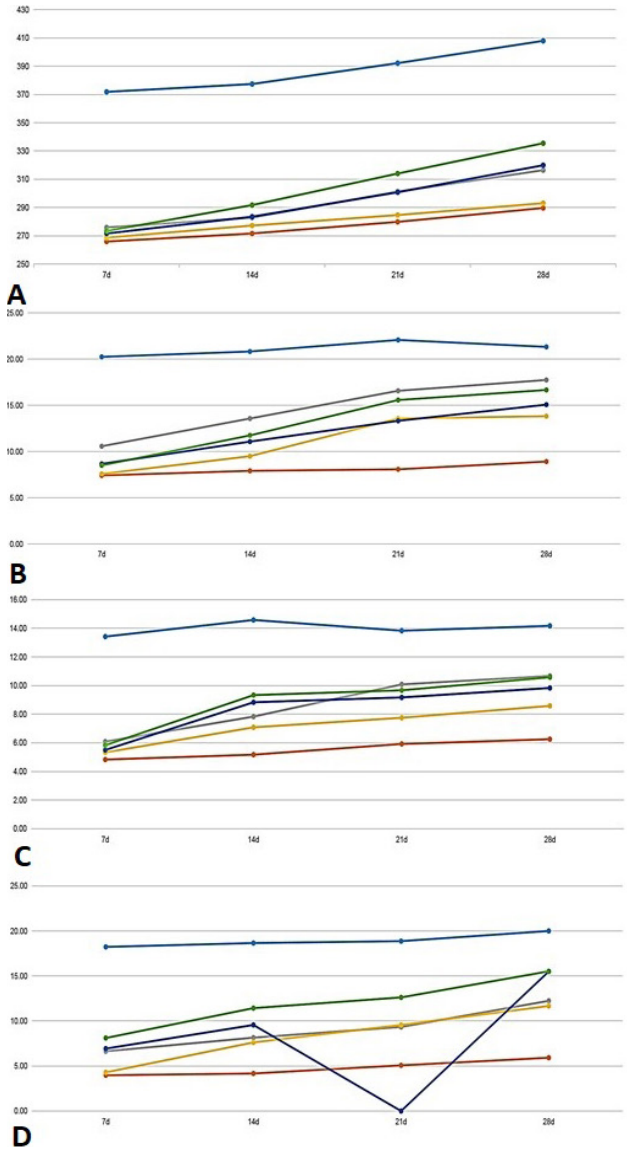

Fig. 1. Effect of Shenxian Congee on body weight (A), horizontal motion (B), vertical motion (C) and fatigue time (D) of rats.

Protein expression of IL-1β and TNF-α in serum

As shown in Table III, on day 28, the levels of the protein concentration of IL-1β in the CFS group and SXC-H group increased compared to those in the CON group ($P < 0.01$). In the FLU, SXC-L, SXC-M and SXC-H groups, the levels decreased compared to those in the CFS group ($P < 0.01$). However, the levels of the protein concentration of IL-1β in the SXC-H group had no significant difference compared to those in the FLU

group. The expression of TNF-α in all treatment groups did not show a significant difference compared to the CON group. The expression of TNF-α in the FLU, SXC-M and SXC-H groups was significantly lower compared to that in the CFS group ($P < 0.01$). However, the SXC-M group and SXC-H group did not have an advantage in decreasing the expression of TNF-α compared to the FLU group.

NF-KB signaling pathway in rats

As shown in Table IV, on day 28, compared with the CON group, the mRNA expression of transforming growth factor β-activated kinase 1 (TAK1), TAB and IKKα in the CFS group was significantly lower ($P < 0.01$); however, the mRNA expression of IκBα, NF-κBp65 and cyclooxygenase-2 (COX-2) did not show the same outcome ($P > 0.05$). The treatment with fluoxetine and the middle and high dosages of SXC could reduce the mRNA expression of TAK1, TAB and IKKα compared with the CFS group ($P < 0.05$ or $P < 0.01$). Meanwhile, the mRNA expression level of IκBα in the SXC-H group was significantly upregulated compared to that in the CFS group ($P < 0.01$), and it revealed more remarkable improvement than it did in the FLU group. On Day 28, the mRNA expression of TAK1 and IκBα in the SXC-H group was significantly upregulated when compared with that in the FLU group ($P < 0.05$ or $P < 0.01$), whereas the mRNA expression of TAB, IKKα and NF-κBp65 did not significantly change ($P > 0.05$). It is worth noting that the middle dosage of SXC could improve the mRNA expression level of COX-2 compared with the CON group only $P < 0.05$); there was no significant difference between the other groups ($P > 0.05$).

Table II. Effect of Shenxian Congee on body weight, horizontal motion, vertical motion, fatigue time of chronic fatigue syndrome rats on day 7, 14, 21 and 28 days (g).

Group	N	7d	14d	21d	28d
Body wt. (g)					
CON	12	371.86±31.36$^{\#\#\ pprr}$	377.41±38.32$^{\#\#pprr}$	392.18±38.62$^{\#\#pprr}$	407.97±40.42$^{\#\#pprr}$
CFS	12	265.94±8.77**	271.63±10.30**	279.86±10.20^{**p}	289.67±10.22^{**pp}
FLU	12	275.87±10.17**	282.91±15.95**	301.23±16.23$^{**\#}$	316.47±17.02$^{**\#\#rr}$
SXC-L	11	268.66±8.11**	277.26±5.41**	284.68±5.56**	293.06±6.62^{**pp}
SXC-M	12	271.64±8.33**	283.51±9.23**	300.81±13.43$^{**\#\#r}$	319.91±14.75$^{**\#\#rr}$
SXC-H	12	273.39±11.16**	291.74±10.12$^{**\#\#rr}$	314.05±10.27$^{**\#\#rr}$	335.49±20.57$^{**\#\#rr}$
Horizontal motion (Mean±SD)					
CON	12	20.25±5.58$^{\#\#pprr}$	20.83±3.16$^{\#\#pprr}$	22.08±4.64$^{\#\#prr}$	21.33±3.39$^{\#\#\ rr}$
CFS	12	7.42±1.68^{**pp}	7.92±1.56^{**pp}	8.08±2.39^{**pprr}	8.92±3.20^{**ppr}
FLU	12	10.58±1.62$^{**\#\#rr}$	13.58±2.78$^{**\#\#rr}$	16.58±4.12$^{\#\#}$	17.75±3.41$^{\#\#}$
SXC-L	11	7.58±1.83^{**pp}	9.50±1.78^{**pp}	13.58±2.39$^{**\#\#}$	13.83±3.59$^{**\#}$
SXC-M	12	8.67±2.27**	11.08±2.64$^{**\#}$	13.33±3.58$^{**\#\#}$	15.08±3.53$^{**\#\#}$
SXC-H	12	8.51±2.08$^{**\#}$	11.75±2.42$^{**\#\#}$	15.58±3.23$^{*\#\#}$	16.67±3.89$^{*\#\#}$
Vertical motion (Mean±SD)					
CON	12	13.42±3.12$^{\#\#pprr}$	14.58±3.82$^{\#\#pprr}$	13.83±3.13$^{\#\#prr}$	14.17±3.56$^{\#\#rr}$
CFS	12	4.83±2.25**	5.17±1.27^{**pp}	5.92±2.35^{**pp}	6.25±2.05^{**pp}
FLU	12	6.08±1.93**	7.83±1.85$^{**\#\#}$	10.08±1.98$^{\#\#}$	10.67±2.42$^{\#\#}$
SXC-L	11	5.33±1.56**	7.08±1.78**	7.75±1.86**	8.58±1.31$^{**\#}$
SXC-M	12	5.50±2.43**	8.83±1.53$^{**\#\#}$	9.17±3.01*	9.83±1.85$^{*\#\#}$
SXC-H	12	5.83±2.52**	9.33±1.61$^{**\#\#}$	9.67±1.23$^{*\#\#}$	10.58±2.11$^{\#\#}$
Fatigue time (Mean±SD)					
CON	12	18.23±0.71$^{\#\#pprr}$	18.66±0.90$^{\#\#pprr}$	18.87±0.78$^{\#\#pprr}$	20.00±0.98$^{\#\#pprr}$
CFS	12	3.97±0.60^{**pp}	4.17±0.76^{**pprr}	5.07±0.85^{**pprr}	5.92±0.94^{**pprr}
FLU	12	6.64±1.70$^{**\#\#rr}$	8.15±2.15$^{**\#\#}$	9.34±1.54$^{**\#\#}$	12.24±1.94$^{**\#\#}$
SXC-L	11	4.29±0.69^{**pp}	7.62±1.16$^{**\#\#}$	9.56±0.95$^{**\#\#}$	11.67±1.48$^{**\#\#}$
SXC-M	12	6.93±0.74$^{**\#\#rr}$	9.56±1.25$^{**\#\#r}$	12.12±1.79$^{**\#\#pprr}$	15.50±1.54$^{**\#\#pprr}$
SXC-H	12	8.11±1.29$^{**\#\#rr}$	11.43±1.56$^{**\#\#pprr}$	12.62±1.28$^{**\#\#pprr}$	15.52±2.01$^{**\#\#pprr}$

Con, control; CFS, chronic fatigue syndrome group; FLU, fluoxetime hydrochloride group; SXC-L, low dose Shenxian congee group; SXC-M, medium dose Shenxian congee group; SXC-H, high dose Shenxian congee group. Compared with CON group ($*p<0.05$, $**p<0.01$), compared with CFS group ($\#p<0.05$, $\#\#p<0.01$), compared with FLU group ($^{p}p<0.05$, $^{pp}p<0.01$), and compared with SXC-L group ($^{r}p<0.05$, $^{rr}p<0.01$).

Table III. Data of ELISA assay of each group on day 28 were presented as mean±SD.

Group	N	IL-1β	TNF-α
CON	12	$3.94\pm0.29^{\#\#}{}_{pprr}$	$27.93\pm5.33^{\#\#}$
CFS	12	$6.38\pm0.47^{**}{}_{pprr}$	$32.94\pm4.59^{**}{}_{pp}$
FLU	12	$3.98\pm0.47^{\#\#}{}_{rr}$	$26.82\pm4.79^{\#\#}{}_{rr}$
SXC-L	11	$4.57\pm0.42^{\#\#}{}_{pp}$	30.74 ± 3.94
SXC-M	12	$4.69\pm0.77^{\#\#}{}_{pp}$	$25.59\pm3.31^{\#\#}{}_{rr}$
SXC-H	12	$4.12\pm0.51^{**\#\#}$	$25.60\pm3.45^{\#\#}{}_{r}$

For abbreviation and statistical detail, see Table II.

DISCUSSION

Malnutrition is a common concomitant symptom of CFS. Due to the influence of fatigue, CFS patients subjective activity intention decreased, physical activity decreased, sleep disorders, etc., often lead to decreased appetite and nutritional intake. Thus, malnutrition occurs, resulting in a decline in immune function. Therefore, paying attention to the nutritional status of CFS patients can delay the development of CFS to some extent. Studies have shown that the addition of nutrients in daily diet can alleviate the fatigue symptoms and psychological status of CFS patients, improve their activity ability and improve their quality of life (Bjørklund *et al*., 2019). Studies have suggested that nutritional status is mainly reflected in appetite and weight. The decrease of appetite and weight loss are the predictors of fatigue. The more obvious the decrease of appetite is, the faster the weight loss is, and the higher the degree of fatigue is (Harrison *et al*., 2019). The results of this study showed that medicinal diet congee (SXC) had a certain protective effect on the recovery of nutritional status of CFS rats, which could promote the growth of appetite in rats, so it could achieve positive results in increasing food intake, water intake and body weight.

Although the causes of CFS are not yet clear, inflammatory cytokines play an important role in the immune response and immune abnormalities of CFS. IL-1B and TNF-α are both pro-inflammatory cytokines of the body and important effectors in the process of inflammatory injury. Corbitts study found that the levels of IL-1B, TNF-α and other pro-inflammatory cytokines in peripheral blood of CFS patients were significantly increased, and were positively correlated with the severity of the disease (Corbitt *et al*., 2019). It can be seen that the increase of pro-inflammatory cytokines may be an important factor in the pathogenesis of CFS. The results of this experiment showed that compared with the CON group, the serum IL-1B andTNF-α concentrations of rats in the CFS group were increased ($P < 0.05$). It has been reported that serum IL-1β and TNF-α in CFS patients were significantly increased (Raijmakers *et al*., 2019). Yangs study also found that the levels of IL-1B and TNF-α in rats continued to increase after exhaustive swimming, which was consistent with the experimental results (Yang *et al*., 2020). It is suggested that the increased secretion of proinflammatory cytokines IL-1β and TNF-α may be closely related to the pathogenesis of CFS. After SXC and fluoxetine hydrochloride treatment, compared with the model group, the serum levels of I-1β and TNF-α in each intervention group were significantly decreased ($P < 0.05$). Thus, SXC and fluoxetine hydrochloride can significantly reduce the concentration of IL-1B, TNF-α.

Studies have shown that TAK1 is one of the central genes activated by NF-κB pathway (Weng and Koh, 2017). IκB kinase (IKK) is an enzyme complex involved in spreading cellular responses to inflammation. It contains two kinase subunits, IKKα and IKKβ, known as the main kinase activated by NF-κB (Colomer *et al*., 2019). Recent studies on IKK gene disruption in mice have shown their importance in mammalian development. For example, a previous study showed that mice lacking IKKα had certain defects in activating NF-κB pathway (Sun, 2012). In this experiment, compared with the CON group, the mRNA expressions of *TAK1, TAB, IKKa, IKBa* and *NF-KBp65*

Table IV. Data of mRNA expression of NF-KB Signaling Pathway of each group on day 28 were presented as mean± SD.

Group	N	TAK1	TAB	IKKα	IκBα	NF-κBp65	COX-2
CON	12	$1.00\pm0.07^{\#\#}{}_{rr}$	$1.01\pm0.11^{\#\#}{}_{rr}$	$1.01\pm0.15^{\#\#}{}_{prr}$	1.02 ± 1.93^{r}	$1.00\pm0.06^{\#\#}{}_{rr}$	1.03 ± 0.25
CFS	12	$1.91\pm0.16^{**}{}_{pp}$	$3.31\pm0.79^{**}{}_{pp}$	$2.35\pm0.26^{**}{}_{pp}$	1.09 ± 0.19	1.65 ± 0.34^{p}	1.29 ± 0.74
FLU	12	$1.08\pm0.26^{\#\#}{}_{rr}$	$1.31\pm0.21^{\#\#}{}_{rr}$	$1.45\pm0.27^{*\#\#}{}_{rr}$	0.85 ± 0.34^{rr}	$1.24\pm0.31^{\#}{}_{r}$	1.44 ± 0.57
SXC-L	11	$1.64\pm0.21^{**}{}_{pp}$	$2.67\pm1.65^{**}{}_{pp}$	$2.04\pm0.47^{**}{}_{pp}$	$1.31\pm0.14^{*}{}_{pp}$	$1.65\pm0.42^{**}{}_{p}$	1.26 ± 0.42
SXC-M	12	$1.55\pm0.46^{**\#}{}_{pp}$	$1.77\pm0.49^{\#\#}$	$1.90\pm0.56^{**\#}{}_{p}$	1.26 ± 0.07^{pp}	$1.56\pm0.22^{**}$	$1.67\pm0.48^{*}$
SXC-H	12	$1.43\pm0.14^{**\#\#}{}_{p}$	$1.50\pm0.24^{\#\#}{}_{r}$	$1.64\pm0.15^{**\#\#}$	$1.53\pm0.35^{**\#\#}{}_{pp}$	$1.29\pm0.17^{\#}{}_{r}$	1.14 ± 0.41

For abbreviation and statistical detail, see Table II.

in the CFS group were significantly increased ($P < 0.01$), suggesting that there was an abnormal expression of NF-KB signaling pathway in CFS rats. The study of Mandarano shows that the increase of NF-xB is one of the key pathological mechanisms of CFS (Mandarano *et al*., 2020). NF-xB can induce the production of pro-inflammatory cytokines, increase glycolysis and further damage the mitochondrial function, leading to mitochondrial failure, thereby damaging the balance of metabolism in the body and presenting fatigue-related symptoms (Simonato *et al*., 2021). After intervention, compared with CFS group, the mRNA expression of TAK1, TAB, IKKa, IKBa and NF-KBp65 in serum of rats in SXC-M group, SXC-H group and FLU group were significantly decreased ($P < 0.05$), indicating that middle and high doses of SXC and fluoxetine hydrochloride have the effect of down-regulating the mRNA expression of the above NF-kB signaling pathway targets, suggesting that middle and high doses of SXC and fluoxetine hydrochloride may play a role in the treatment of CFS by inhibiting NF-KB signaling pathway.

In short, medicated diet Shenxian Congee can relieve fatigue symptoms of CFS rats, improve the general situation and weight, improve exercise capacity. It can reduce the concentration of inflammatory factors IL-1β and TNF-α in CFS rats, and play an immune regulatory function. The NF-KB signaling pathway of CFS rats was activated, and the expression of target protein mRNA in the pathway was down-regulated, which played a certain role in the prevention and treatment of CFS. In the future, we can further explore the effect of medicated Shenxian Congee on NF through the specific inhibitor of protein kinase- κ B signaling pathway related protein expression.

Limitations

Due to the short intervention time in this study, the long-term nursing effect of medicinal diet Shenxian Congee was not observed. In addition, this study will focus on NF- κB signal pathway was used as the research pathway to observe the mechanism of herbal diet Shenxian Congee in regulating CFS rats. However, there may be the interaction of multiple signal pathways. Based on the existing problems, the experimental design will be improved in the future research, the experimental cycle will be extended appropriately, and the long-term effect of medicated diet will be observed as much as possible.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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