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THE ANIMAL MODEL ESTABLISHMENT METHODS AND MODEL EVALUATION FOR LIVER STAGNATION AND SPLEEN DEFICIENCY SYNDROME

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Abstract: The fast pace of modern life has increased stress, leading to a faster incidence of liver qi stagnation and spleen deficiency syndrome in diseases. Therefore, liver qi stagnation and spleen deficiency syndrome has become a common clinical syndrome. Animal models of liver qi stagnation and spleen deficiency syndrome are used in various disease areas, and numerous methods exist for evaluating these models, resulting in problems such as confusion regarding counter-evidence formulas. This article mainly evaluates the modeling methods, behavioral testing methods, and counter-evidence formulas for liver qi stagnation and spleen deficiency syndrome in various diseases, providing theoretical support for experimental research on this syndrome.
Keywords: Liver qi stagnation and spleen deficiency syndrome; Animal model; Counter-evidence formula; Behavioral science; Tail clamping; Restraint

1 INTRODUCTION

With the increasingly rapid pace of modern life and rising levels of stress in both work and study, the incidence of liver stagnation and spleen deficiency syndrome has shown a marked upward trend. As clinical research must be grounded in solid basic research, it is essential to establish reliable animal models of liver stagnation and spleen deficiency syndrome and translate the findings into clinical practice. Since their initial application in digestive system disorders such as gastritis and liver cancer, these animal models have gradually been extended to a broader range of diseases, including tic disorder and depression [1,2]. Over more than 30 years of continuous refinement and innovation, the modeling system has become increasingly sophisticated; however, challenges remain—particularly regarding the accuracy of behavioral indicators and the lack of standardized medicinal prescriptions following model establishment. To gain a clearer understanding of current research progress, this study retrieved literature from CNKI published before February 1, 2020, using the topic search terms “liver stagnation and spleen deficiency + animal model.” A total of 267 relevant articles were identified, from which 26 representative studies were selected for detailed review and discussion.

2 APPLICATION OF ANIMAL MODELS OF LIVER STAGNATION AND SPLEEN DEFICIENCY SYNDROME IN VARIOUS DISEASES

Animal models of liver stagnation and spleen deficiency syndrome have been widely used across multiple disease areas. The selection of experimental animals varies considerably in terms of species, weight, and age, while the primary modeling methods include tail-clamping stimulation, restraint stress, and combined modeling approaches. Modeling duration, detection indicators, and observable macroscopic features differ according to disease type. These key characteristics and their applications are summarized in Table 1.

Table 1 Application of Liver Qi Stagnation and Spleen Deficiency Syndrome in Various Systems

Disease	animal models	Weight and age	Modeling methods	Molding time	detection indicators	Macroscopic representation
Irritable bowel syndrome [3]	SD pregnant mice Pregnancy duration: 18.2 ± days	-	Mother-infant separation	9 weeks	D-xylose, serotonin (5-HT) , brain-derived neurotrophic factor, immunoglobulin A, immunoglobulin G	Reduced activity, sluggish response, and clustering
Fatty liver [4]	Wistar male rats	160-200 g	20% ethanol solution administered by gavage	2 weeks	Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase, total cholesterol (GGT), triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.	Irritability, decreased activity, loss of appetite, yellowish coat, loose stools, and a few bruises on the tail.
Liver injury [5]	Wistar male rats	180-220 g	Gavage with ethanol	1 week	ALT, AST, prealbumin, total bilirubin, total bile acids	Laziness, weight gain
Duck hepatitis B virus infection [6]	Guangzhou Ma Duck	40-50 g 1 day old	Restraint, dietary disorder	2 weeks	duck hepatitis B virus	listless, with sparse downy hair, irritable, and lacking appetite.
Liver cancer [7]	Wistar rats	100-200 g	Restraint, dietary disorder	9 weeks	T lymphocytes, inflammatory cytokine-2 (IL-2) , and tumor necrosis factor -γ (TNF-γ)	Irritability, anger, weakness, lack of exercise, loose stools, dry and yellow hair
Depression [8]	SD rats, male	180-200 g 6-8 months	Restraint, tilted cage, swimming, tail clamping, shaking the cage, water restriction, and odor stimulation.	7 weeks	IL-6, TNF-α	Reduced appetite and decreased physical condition
Nervous system [9]	Wistar male rats	240 ± 20g	Electrical stimulation	9 days	Hypothalamic CRF content, locus coeruleus CRF content	Avoidance, reduced activity, irritability
Tourette syndrome [10]	SD rats, male	40-60g 3 weeks old	Restraint, swimming, and dietary indiscretion	3 weeks	Gastrin, D-xylosin	Decreased appetite, low mood, increased bowel movements
Tinnitus [11]	Wistar male rats	222-238 g 6-8 weeks old	Restraint, swimming, and dietary indiscretion	20 days	False positive reaction	Disheveled hair, slow movement, huddling together, and closed eyes
Chronic pharyngitis [12]	Wistar male rats	180-220 g	Restraint, dietary disorder	3 weeks	5-HT1B, 5-HT2C	Loose hair, occasional loose stools, low activity level, and dirty anus.

3 BEHAVIORAL TESTING METHODS

3.1 Open Field Experiment

Yue Lifeng et al. constructed a custom open-field apparatus measuring 1 m × 1 m × 40 cm, with a camera positioned at the center of the base. All experiments were conducted in a dark, enclosed environment. Each rat was placed in the central area of the box, and its behavior was recorded and timed simultaneously. The animal's activity was observed for 5 minutes. After each trial, the rat was removed and the apparatus was thoroughly cleaned to eliminate any residual odor that might influence subsequent observations. Behavioral indicators included the total number of grid crossings, the frequency of rearing, and the frequency of grooming behaviors (such as scratching, washing the face, and licking the paws) [13].

Similarly, Zhao Bo et al. designed an open-field box measuring 122 cm in length and 45 cm in height. The bottom was divided into 16 equal-area squares, and each rat was placed at the center of the apparatus. The number of squares crossed served as the activity score, and the behavioral indicators were consistent with those used by Yue Lifeng et al.

[14].

3.2 Sucrose Preference Test

Sucrose preference percentage is widely recognized as an objective and reliable indicator for evaluating anhedonia and reduced reward sensitivity. Liu Yuan et al. fasted rats for 24 hours and then provided each animal with 200 mL of 1% sucrose solution and an equal volume of sterile water in a quiet environment. After 24 hours of free drinking, the consumption of each liquid was recorded, and the sucrose preference percentage was calculated [15]. Liu Yueyun et al. conducted a similar assessment but first trained the rats to develop a preference for 1% sucrose solution for two consecutive days, beginning three days before the completion of modeling. On the third day, following a fasting period, the rats were allowed access to both sucrose water and pure water for 1 hour, after which the intake of each was measured to determine sucrose preference [16].

3.3 Forced Swimming Experiment

Zhou Guoer et al. conducted the forced swimming test by placing mice in a container filled with 25-cm-deep water maintained at 25 °C. Each mouse was allowed to swim for 6 minutes. Immobility was defined as the state in which the mouse floated with minimal limb movement and ceased active struggling for at least 3 seconds. The cumulative immobility time during the final 4 minutes of the test was recorded and used as the primary behavioral indicator [17].

3.4 Elevated Plus Maze Test

The elevated plus maze was positioned 50 cm above the ground and consisted of two open arms, two closed arms, and a central platform, with dimensions of 45 cm × 15 cm for the open arms, 45 cm × 15 cm × 30 cm for the closed arms, and 15 cm × 15 cm for the central platform. The experiment was conducted in a quiet environment, and the maze was kept dark prior to behavioral recording. Each rat was placed on the central platform facing one of the closed arms, and its activity was monitored for 5 minutes. Behavioral indicators included the number of entries into the open arms and the total time spent in these arms. After each session, the apparatus was thoroughly cleaned to remove odor cues [18].

3.5 Tail Suspension Test

Zhang Beihua et al. performed the tail suspension test in a quiet, disturbance-free environment. Each rat was suspended by its tail and allowed to hang freely, and the duration of complete immobility within a 5-minute observation period was recorded as the primary behavioral indicator [19].

4 COUNTER-EVIDENCE PRESCRIPTIONS

4.1 Xiaoyao Pills

After artificial modeling, the solution was adjusted to a concentration of mL^{-1}, and each rat received an oral dose of 3.24 $mL \cdot kg^{-1} \cdot d^{-1}$. Following administration, the liver volume decreased and the tissue became softer compared with untreated rats exhibiting liver stagnation and spleen deficiency. In addition, Xiaoyao Pills were shown to improve blood lipid profiles and alleviate disturbances in glucose and amino acid metabolism in rats [20].

4.2 Tongxie Yaofang

After establishing the model, Wang Min et al. administered Tongxie Yaofang to rats by gavage at doses equivalent to 4, 8, and 16 times the adult dosage. Following 21 days of treatment, rats in the Tongxie Yaofang groups showed a marked attenuation of body-weight loss and a significant reduction in gastric mucosal injury associated with liver stagnation and spleen deficiency syndrome [21].

4.3 Chaihu Shugan San Combined with Sijunzi Tang (Chai Shu Si Jun Zi Tang)

After establishing the model, Li Cong et al. administered the drug to rats at a dose of 3.57 g/kg for two weeks. The results indicated that Chai Shu Si Jun Zi Tang exerted a noticeable ameliorative effect on the abnormal hemorheology observed in rats with liver stagnation and spleen deficiency syndrome [22].

5 CONCLUSION

Animal models of liver stagnation and spleen deficiency syndrome are primarily established using rats and mice, with few studies employing large animals. As a result, key diagnostic features in traditional Chinese medicine (TCM)—such as tongue appearance, pulse, and facial expression—cannot be observed. In addition, inconsistencies in the sex, age, and body weight of experimental animals may influence modeling outcomes [23,24]. Therefore, when replicating this model, the selection of animals should align with the experimental objectives, considering anatomical and physiological characteristics, and their age and weight should be standardized.

A second challenge is the lack of standardized criteria for evaluating model-inducing stimuli. Techniques such as restraint stress and tail clamping not only lack quantifiable stimulation intensity but may also cause physical injury, thereby influencing subsequent behavioral assessments. Moreover, after modeling, distinguishing between liver stagnation alone and liver stagnation combined with spleen deficiency is often difficult.

From a modern biomedical perspective, animal models of liver stagnation and spleen deficiency involve functional disturbances across multiple systems—including the nervous, digestive, and immune systems—which complicates the identification of specific biomarkers. Beyond commonly measured indicators such as AST, ALT, gastrin, and 5-HT, physicochemical markers reported in the literature remain inconsistent. During model evaluation, syndrome classification relies heavily on macroscopic signs; however, liver qi stagnation and spleen qi deficiency represent two major TCM syndrome groups that often overlap. Furthermore, differentiating spleen qi deficiency from spleen yang deficiency is challenging, leading to confusion and variability in macroscopic assessments after modeling [25].

Counter-evidence prescriptions play a crucial role in validating whether a model has been successfully established. Yet, current practice remains problematic: the selection of formulas is often arbitrary, the variety is overly broad, and in many cases, counter-evidence prescriptions are not used at all after modeling. This approach deviates from the TCM principle of integrating theory, method, formula, and medicine as a unified system [26]. Additionally, behavioral testing methods vary widely in terms of environmental requirements and apparatus specifications, raising concerns about the reliability and comparability of experimental results. Therefore, it is essential to standardize both counter-evidence prescriptions and behavioral testing methods to ensure the accurate and scientifically sound verification of model success.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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