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The recovery effect of Feixingcao tea (*Teucrium viscidum* Bl.) on inhibiting the decreased exercise ability caused by chronic alcoholic liver injury

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Abstract

Feixingcao tea is a health tea, and this study aimed to investigate its improvement in the decreased exercise capacity caused by chronic alcoholic liver injury. A mouse model of chronic liver injury was established by gavage of alcohol, and Feixingcao tea extract (FXCTE) was used to observe the exercise ability, pathological sections and molecular biology methods to detect the effect of FXCTE. And the active ingredient of FXCTE is detected by HPLC. Experimental results showed that FXCTE could increase the time of exhaustive running and negative-gravity swimming in mice with liver injury. FXCTE could reduce alcohol-induced liver lesions. FXCTE could reduce the levels of Lactic acid, BUN, GOT, GPT, TNF-α and iNOS in serum of mice with liver injury. FXCTE could also up-regulate the mRNA expression of Cu/Zn-SOD, Mn-SOD, CAT and down-regulate the expression of nNOS, syncytin-1 in liver injury mice. Component analysis showed that FXCTE contained mangiferin, rutin, ferulic acid, isochlorogenic acid B, astragalin, rosmarinic acid. FXCTE is a health-care tea containing a variety of active compounds, which can improve chronic alcoholic liver damage and improve the decline in exercise ability caused by liver damage.

Keywords: Feixingcao tea; chronic alcoholic liver injury; exercise ability; oxidation; liver.

Practical Application: Long-term drinking can cause a series of discomforts in the human body, including the decline of body function and the decline of exercise ability. Functional tea Feixingcao tea has the effect of reducing alcohol damage and improving exercise ability, and can be eaten as a health food for hangover.

1 Introduction

Drinking, as a habit of human beings for thousands of years, has always been accompanied in life. According to the World Health Organization's global alcohol and health status report, 2.3 billion people aged 15 and above are drinking, accounting for nearly one third of the global population. The report of the World Health Organization shows that "there are more than 3 million alcohol-related deaths worldwide every year". The report also showed that people who died from alcohol-related causes accounted for 5.3% of global deaths, higher than the 2.8% of diabetes (World Health Organization, 2018). About 90% of the alcohol ingested by the human body is metabolized in the liver, and poor alcohol metabolism is the main cause of alcoholic liver disease (Zhang et al., 2015). The liver organ in the human body is our detoxification system. Once the function of the liver organ is damaged, it is easy to accumulate a lot of toxins in the body, which will have a great impact on the body (Ding et al., 2022). When the human body is exercising, the body will accelerate the consumption rate of energy glycogen, and some of the energy glycogen is transferred from the liver organ through the blood in the body, so when the energy glycogen in the liver organ If enough, it can continue to provide energy to the human body, so that the muscles of the human body have always been active. Exercise and the health of the liver organs are closely related and interrelated. Exercise can improve the function of the liver organs, and in turn, a healthy liver can also improve the exercise capacity of the human body (Zhao et al., 2022).

Teucrium viscidum Bl. is a plant distributed in China, Japan, Korea, Myanmar, India, Indonesia, Philippines. Which is used as health tea and traditional Chinese medicine in China, and it is called Feixingcao tea. Drinking Feixingcao tea for a long time is considered to have the effect of relieving rheumatoid arthritis, bruise injury, lung abscess, acute gastroenteritis, indigestion and frostbite swelling and pain (Tao et al., 2019). Some studies have shown that some health teas have special functional ingredients that can alleviate liver damage caused by alcohol and protect the body. The main effects of these health teas on the liver are believed to reduce the oxidative stress damage and inflammatory response of the liver caused by alcohol (Wang et al., 2015; Wang et al., 2019; Zhan et al., 2022). In this study, an animal model of liver injury was established by alcohol, and the recovery effect of Feixingcao tea on the exercise capacity of mice with liver injury was observed, and its mechanism of action was preliminarily studied.

2 Materials and methods

2.1 Feixingcao tea aqueous solution extract

According to the general practice of tea citations, Feixingcao tea (Bozhou Guqingtang Pharmaceutical Co., Ltd., Baozhou,

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Anhui, China) was extracted with boiling water of 100 °C. 500 g of Feixingcao tea was added to 4.5 L of distilled water, heated to 100 °C, kept at the temperature and continuously stirred and extracted for 30 min. The water-soluble extract was filtered and lyophilized to obtain the Feixingcao tea extract (FXCTE).

2.2 Animal experiment

ICR male mice (n = 50, body weight: 23 ± 2 g, Experimental Animal Center of Xi'an Jiaotong University Medical College, Xi'an, Shaanxi, China) that were six weeks old were kept in a sterile setting with a temperature of 25 °C and a relative humidity of 50% on a 12-hour light/dark cycle. Standard mouse food and drinking water were eaten ad libitum, and litter was changed every two days for a week of adaptive upbringing. Ten mice each were randomly assigned to one of four groups: normal group, model group, FXCTE low concentration group (FXCTE-L), and FXCTE high concentration group (FXCTE-H). The mice in the control group were given distilled water twice daily, with a 12-hour gap between each dose, starting at the beginning of the trial. The other three groups of mice were gavaged with 40% alcohol (v/v) twice a day, 0.1 mL/10 g b.w. each time, with a 12-hour interval between the two times, and the gavage of distilled water and alcohol lasted for 4 weeks. Then the mice in the normal group and the model group were continuously gavaged with distilled water for 4 weeks, and the FXCTE-L and FXCTE-H mice were gavaged with FXCTE at a concentration of 50 and 100 mg/kg b.w. daily for 4 weeks. After the above experiments are completed, follow-up measurements are carried out. The Shaanxi Police College Ethics Committee authorized the experimentation procedure (Xi'an, Shaanxi, China).

2.3 Running exhaustion test in mice

The exercise exhaustion test for mice was performed on the second day after the gavage sample experiment was finished, and the mice's exercise fatigue time was tracked and assessed. The mice were compelled to use the treadmill for exercise. The mice all displayed high-frequency shortness of breath because they were unable to keep up with the treadmill's set pace and their rear limbs were pulled by the belt for more than 30 s (YH-CS, Wuhan Yihong Technology Co., Ltd, Wuhan, Hubei, China). There was no obvious response after physical stimulation (Wu et al., 2022). The time at this time was recorded.

2.4 Swimming experiment of mice with negative weight to exhaustion

The mice were placed in a constant temperature water tank (28 ± 2 °C), and the water flow speed in the water tank was maintained at 2 m/s. The swimming state of the mice was observed. The mice could not swim and sink to exhaustion for more than 3 s. The swimming time of the mice was recorded (Yi et al., 2021). At the same time, the mice were fished out to rest.

2.5 Tissue H&E staining analysis

The liver tissues with a diameter of around 1.5 cm were cut out and preserved in a 10% formalin solution after the mice's internal organs had been removed and the blood stains had been cleaned with normal saline. The preserved liver tissue was dried in a gradient of ethanol, clarified in a 30-minute soak in xylene and ethanol, embedded in paraffin, sectioned into sections of about 2-3 m, and mounted on glass slides. Finally, it was completed using hematoxylin and eosin (H&E) dyes, and under a light microscope, morphological alterations were checked for (BX53, Olympus, Tokyo, Japan).

2.6 Serum routine index detection

The blood was drawn from the dissected mice, centrifuged at 4000 rpm for 10 min at 4 °C, and the serum was then separated and collected. Then, using a biochemical kit approach, the serum levels of lactic acid, BUN, GOT, and GPT were determined (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.7 Enzyme linked immunosorbent assay of serum markers in mice

The method of collecting mouse serum in method 2.7 was continued, and then the levels of inflammation-related cytokines TNF-α and iNOS (Thermo Fisher Scientific, Waltham, MA, USA) in mouse serum were determined according to the ELISA instructions (Bian et al., 2022).

2.8 Real-time quantitative PCR detection

It was discovered how the mRNA was expressed in the skeletal muscle and liver tissues of mice. Mouse liver and skeletal muscle tissue were excised and minced, and total RNA was extracted from the tissues using the Trizol reagent (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). To create cDNA templates, reverse transcription was carried out using the Revert Aid First Strand cDNA Synthesis Kit. Then, using a StepOnePlus real-time PCR machine (Thermo Fisher Scientific, Waltham), a mixture of 10 μL SYBRGreen PCR Master Mix, 1 μL upstream primer and 1 μL downstream primer, 1 μL cDNA template, and 7 μL DEPC was amplified. The relative expression level of each gene was then determined using the $2^{-\Delta\Delta CT}$ technique, where CT stood for cycle threshold and β-actin was utilized as an internal reference gene (Hu et al., 2022; Long et al., 2022).

2.9 Analysis of components by high performance liquid chromatography

The diluted samples were loaded by an autosampler and analyzed in a high performance liquid chromatograph (UltiMate 3000, Thermo Fisher Scientific). The chromatographic conditions were: Accucore C18 column (2.6 μm, 4.6 × 150 mm), mobile phases A and B were 0.5% acetic acid water and acetonitrile, respectively, the detection flow rate was 0.8 mL/min, and the detection wavelength was 359 nm.

2.10 Statistical analysis

For each mouse's experimental measurement indicators, three or more parallel tests were performed, and the average value was obtained. IBM SPSS 22 statistical software was used to examine the data. The mean standard deviation of the

experimental outcomes is given (SD). One-way ANOVA with Duncan's multiple range test was used to analyze differences between the means of each group (MRT). Differences with a $p < 0.05$ significance level were judged statistically significant.

3 Results

3.1 Running to exhaustion in mice

Figure 1 shows that the normal group's fatigue running time was the longest (48.7 min), while the model group's was the smallest (31.6 min). FXCTE may improve the fatigue running time of mice with liver damage. The FXCTE-H group's fatigue running time (42.5 min) was longer than the FXCTE-L group's (36.7 min).

3.2 Effects of GTE on in vitro survival of primary thyroid cells and SW579 thyroid cancer cells

Similar to the exhaustive running time results (Figure 2), in the negative-gravity exhaustive swimming experiment, the normal group mice also showed the longest swimming time (287.5 s). Swimming time was considerably ($p < 0.05$) shorter in mice with liver damage than in the control group. The swimming time of the mice in the FXCTE-L (205.6 s) and FXCTE-H (241.7 s) groups was greater than that of the model group (165.6 s), and the FXCTE-H was only slightly shorter than that of the normal group.

3.3 Serum levels of lactic acid, BUN, GOT and GPT

The histomorphology of mouse liver tissue is shown in Figure 3. Normal mice had clear and full hepatic lobules, and hepatocytes were dispersed radially with the central vein in the center. The model group's hepatic lobule structure was mainly complete, the liver cells were neatly ordered, the dispersed cells reduced in size, the cytoplasmic density rose, the nucleoplasm was condensed, the nuclear membrane nucleolus was disrupted, and apoptotic bodies were infrequently observed. FXCTE could ameliorate alcohol-induced liver tissue damage, and the improvement effect was proportional to the concentration.

3.4 Serum levels of lactic acid, BUN, GOT and GPT

As indicated in Table 1, the model group had the highest blood levels of BUN, Lactic acid, GOT, and GPT, whereas the normal group had the lowest levels of BUN, Lactic acid, GOT, and GPT. FXCTE could lower BUN, Lactic acid, GOT, and GPT levels in liver damage mice (model group), and the impact of high concentration (FXCTE-H) was larger than that of low concentration (FXCTE-L).

3.5 Serum cytokine levels of TNF-α and iNOS

The serum inflammatory cytokine detection findings revealed that the mice in the model group had the greatest levels of TNF- and iNOS (Figure 4). FXCTE may prevent liver damage while increasing TNF- and iNOS levels. TNF- and iNOS levels may be reduced by FXCTE-H. The levels of inflammatory cytokines in FXCTE-H mice were only slightly higher than in the control group.

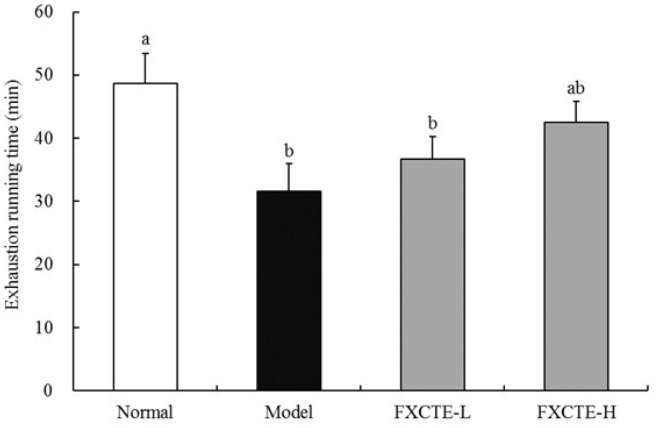

Figure 1. Running time to exhaustion in mice with chronic alcoholic liver injury. a-b: Different lowercase letters indicate significant difference at $p < 0.05$ level.

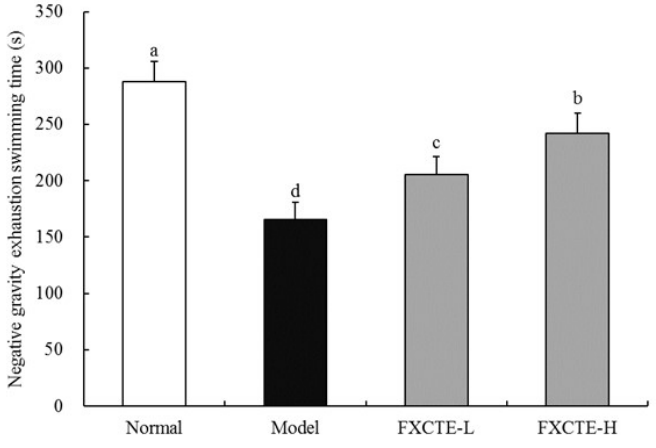

Figure 2. Weight bearing exhaustive swimming time in mice with chronic alcoholic liver injury. a-d: Different lowercase letters indicate significant difference at $p < 0.05$ level.

Table 1. Serum levels of lactic acid, BUN, GOT and GPT in mice with chronic alcoholic liver injury.

Group	Lactic acid (mg/L)	BUN (mg/L)	GOT (U/L)	GPT (U/L)
Normal	0.32 ± 0.03^{d}	132.64 ± 14.33^{d}	15.19 ± 2.87^{d}	8.15 ± 0.72^{d}
Model	1.25 ± 0.12^{a}	288.79 ± 21.05^{a}	94.35 ± 4.51^{a}	78.92 ± 2.68^{a}
FXCTE-L	0.87 ± 0.11^{b}	235.18 ± 19.71^{b}	68.17 ± 3.89^{b}	51.07 ± 3.66^{b}
FXCTE-H	0.59 ± 0.08^{c}	174.36 ± 20.55^{c}	38.53 ± 4.02^{c}	24.38 ± 3.19^{c}

[a-d]Different lowercase letters indicate significant difference at $p < 0.05$ level.

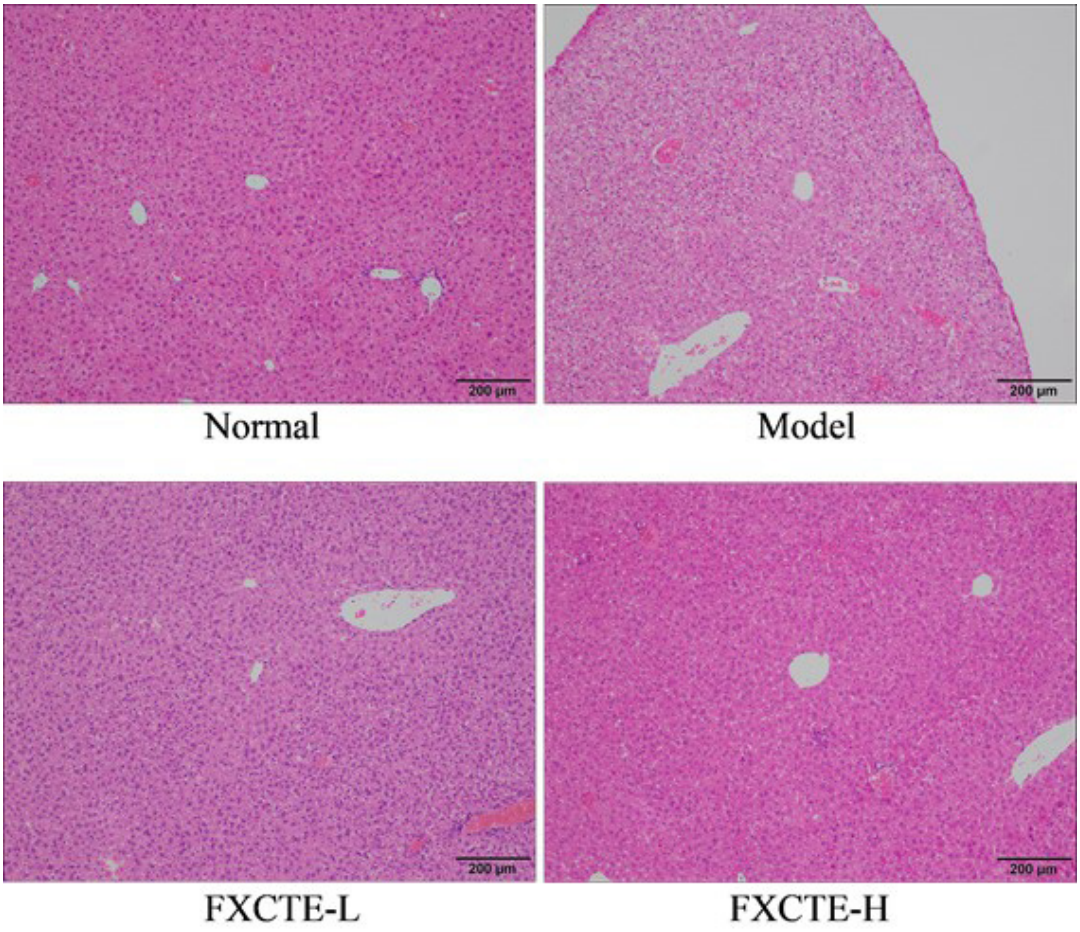

Figure 3. H&E pathological observation of liver tissue in mice with chronic alcoholic liver injury.

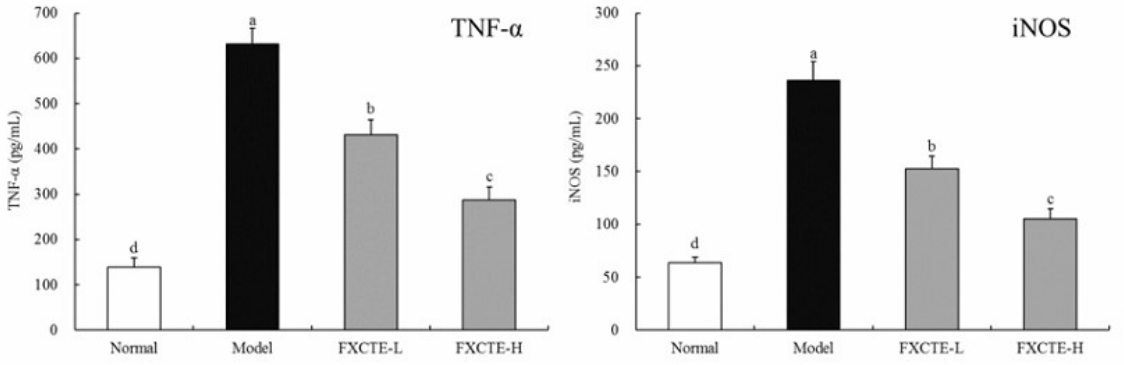

Figure 4. Serum cytokine levels of TNF-α and iNOS in mice with chronic alcoholic liver injury. a-d: Different lowercase letters indicate significant difference at $p < 0.05$ level.

3.6 mRNA expression of Cu/Zn-SOD, Mn-SOD and CAT in mouse liver tissue

Figure 5 shows that the expression of Cu/Zn-SOD, Mn-SOD, and CAT in the liver tissue of the normal group mice was the strongest, while the expression in the model group mice was the smallest. In the model state, FXCTE could up-regulate the expressions of Cu/Zn-SOD, Mn-SOD, and CAT in mice, and the degree of up-regulation increased with concentration.

3.7 mRNA expression of nNOS and syncytin-1 in mouse skeletal muscle tissue

Figure 6 demonstrates that the model group had the highest levels of nNOS and syncytin-1 mRNA expression in mouse skeletal muscle tissue. FXCTE could lower the expression of nNOS and syncytin-1 compared to the model group, and the FXCTE-H group dropped even more, just slightly higher than the normal group.

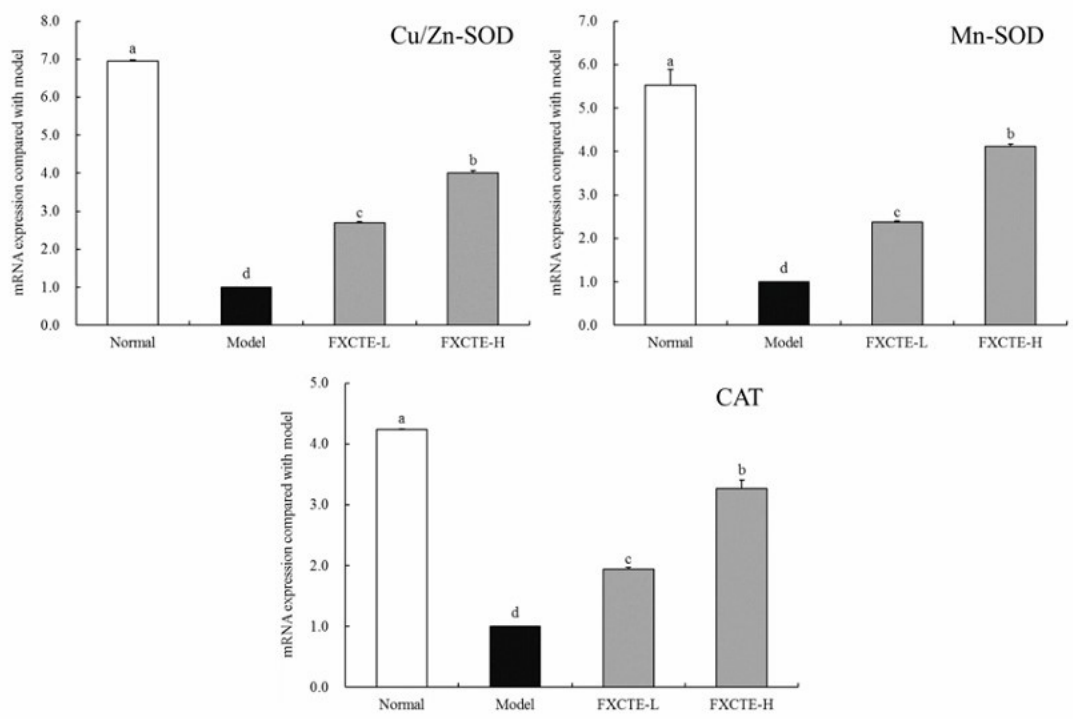

Figure 5. mRNA expression of Cu/Zn-SOD, Mn-SOD and CAT of liver tissue in mice with chronic alcoholic liver injury. a-d: Different lowercase letters indicate significant difference at $p < 0.05$ level.

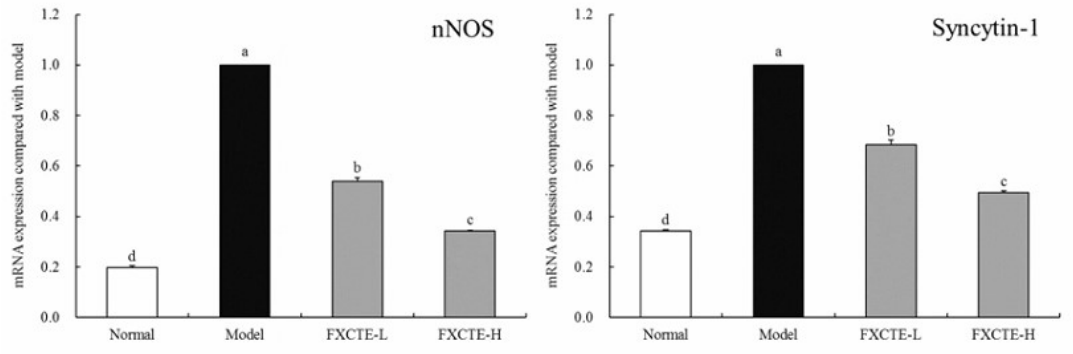

Figure 6. mRNA expression of nNOS and syncytin-1 of skeletal muscle tissue in mice with chronic alcoholic liver injury. a-d: Different lowercase letters indicate significant difference at $p < 0.05$ level.

3.8 Compound composition of FXCTE

After detection by high performance liquid chromatography, the results showed that the samples contained 6 main compounds, which were isomanigiferin, rutin, ferulic acid, isochlorogenic acid B, vetchin and rosmarinic acid (Figure 7). Among them, the content of ferulic acid and astragaloside is the highest

4 Discussion

The time it takes to run or swim to exhaustion is a frequent indication for empirically reflecting exercise ability, and increase in exercise ability is an intuitive representation of the body's illness healing (Zhang et al., 2021). The liver is the central organ of alcohol metabolism, and it is very sensitive to reflect the biological damage caused by alcohol, and it is also the place where free radicals and lipid peroxides are easily generated after damage (Wang et al., 2022). In this study, FXCTE could ameliorate alcohol-induced liver lesions in mice, thereby ameliorating the decreased exercise capacity caused by liver injury.

BUN levels in serum are typically steady under normal conditions. When the body suffers from liver illness, the metabolism of glycogen and fat is unable to fulfill the body's energy demands, and the body must rely on protein degradation for energy supply. As a result, the change in BUN is visible and may be used as a measure to monitor exercise capacity (Hou et al., 2022). Blood lactic acid is an acidic metabolite produced by the body during exercise heat generation. Excessive lactic acid accumulation will

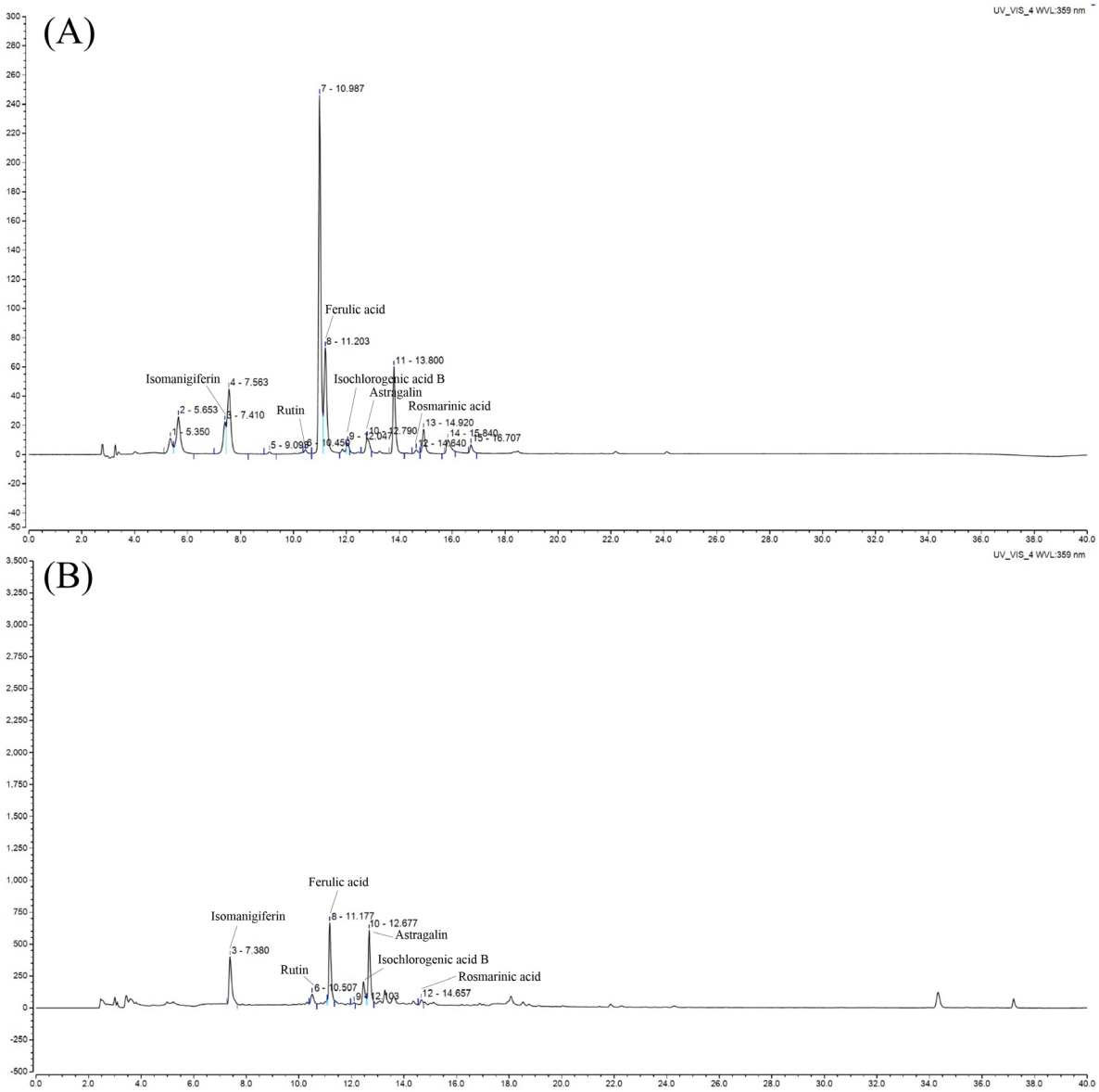

Figure 7. Compound composition of FXCTE, (A) chromatogram of standard, (B) chromatogram of FXCTE.

disrupt the relative stability of the internal environment and the normal metabolic process in the body, resulting in tiredness symptoms such as muscular pains, chills, and headaches. Therefore, blood lactate reflects the body's aerobic capacity and an important indicator of fatigue (Wu et al., 2019). When training in acute failure, hypoxia or hypoxia produces muscle damage and excessive energy consumption, particularly muscle fiber injury or pure mechanical strain, which destroys muscle tissue and causes enzymes in the muscle to seep into the blood, causing GPT and GOT activity to rise. GPT and GOT mainly exist in the liver, and liver damage can have a great impact on the regulation of GPT and GOT, thereby indirectly affecting exercise capacity (Chen et al., 2022). iNOS and TNF-α are involved in a variety of pathological processes in inflammation and immune response, and are important cytokines in inflammatory response. It is obvious when liver damage affects body function (Labsi et al., 2022). FXCTE could modulate BUN, lactate, GOT, GPT, iNOS and TNF-α in mice, thereby improving liver injury and improving exercise capacity in mice.

Cu/Zn-SOD, Mn-SOD, and CAT are all antioxidant enzymes found in the body. Exercise may readily produce oxidative stress in the body and generate a big number of free radicals when the body's ability to exercise declines. These antioxidant enzymes can help to reduce exercise-induced weariness and improve exercise performance by inhibiting free radicals in the body (Yi et al., 2021). A huge quantity of nNOS is located on the fast-twitch fiber membrane, and nNOS expression is dramatically increased following strenuous activity (Shabeeh et al., 2013). After liver injury, the body's metabolism is affected, and after exercise capacity is reduced, syncytin-1 may be highly expressed in skeletal muscle, which may damage motor neurons and further affect exercise capacity. FXCTE improved liver tissue

and skeletal muscle by regulating the expression of the above mRNAs, thereby enhancing exercise capacity.

Isomanigiferin, rutin, ferulic acid, isochlorogenic acid B, astragalin and rosmarinic acid are all important antioxidant and anti-inflammatory compounds with good biological activities (Yuan et al., 2019; Qi et al., 2022; Luo et al., 2022). FXCTE regulated liver function through these active compounds, thereby enhancing motor function and acting as a health tea.

5 Conclusion

Feixingcao tea is a kind of traditional health care tea. In this study, certain scientific researches were carried out on its function, and it was found that it has a good regulating effect on liver damage. And through the liver dysfunction caused by chronic alcohol, the exercise ability of mice is improved. In the future, Feixingcao tea can be used as a health tea to relieve hangover and restore the body's physical and exercise ability.

Abbreviations

FXCTE: Feixingcao tea extract. HPLC: high performance liquid chromatography. qPCR: quantitative polymerase chain reaction. BUN: blood urea nitrogen. GOT: aspartate aminotransferase. GPT: glutamic pyruvic transaminase. iNOS: inducible nitric oxide synthase. nNOS: neuronal nitric oxide synthase. TNF-α: tumor necrosis factor alpha. Cu/Zn-SOD: copper/zinc superoxide dismutase. Mn-SOD: manganese superoxide dismutase. CAT: catalase.

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