ISSN: 2688-8645

Intervention of Vine Tea Extract on Non-alcoholic Fatty Liver in Mice

Kaixuan Kang, Tong Zhou, Mingju Lu, Jiajun Song, Dian Yu, Lixin Qiu,
Jiangyuan Chen and Fahu Yuan*

School of medicine, Jianghan University, Wuhan 430056, China

Abstract

To investigate the effects of vine tea extract (VTE) on high-fat-diet (HFD) induced obesity in mice. A C57BL/6J mouse model of obesity induced by 8-week high-fat diet was established. By measuring the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the contents of triglyceride (TG), total cholesterol (T-CHO), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in serum. Pathological changes were observed by H&E staining to explore the effect of VTE on obese mice. The results showed that, compared with the model group, the levels of AST, ALT, TG, T-CHO, LDL-C in serum were significantly decreased, while the levels of HDL-C in serum was increased in the VTE low and high dose groups. It also improved fat vacuoles, inflammatory infiltration, and inflammatory response in liver tissue. In conclusion, VTE may improve lipid metabolism in HFD-induced obese mice by inhibiting oxidative stress and inflammatory response.

Keywords

Vine Tea Extract; High Fat Diet; Obesity; Oxidative Stress; Inflammatory Response.

1. Introduction

Obesity is a common nutritional disorder, which is characterized by hypertrophy and proliferation of adipocytes in the body and excessive accumulation of body fat[1]. According to statistics, the incidence and mortality of obesity in the world have increased significantly, and there are more than 1 billion overweight people, of which at least 300 million are obese[2]. Obesity is a risk factor for diabetes, coronary heart disease, cerebrovascular disease, hypertension, hyperlipidemia and other diseases, which plays a role in the pathogenesis of the above diseases, inducing factors, aggravating factors or both[3]. Although the etiology of obesity is complex, dietary factors, especially the consumption of high-fat diet, are the main risk factors for the development of obesity[4]. Long-term intake of high-fat and high-calorie diet will affect the body's normal lipid metabolism, resulting in disorders of fat decomposition, synthesis and storage, leading to abnormal accumulation of body fat.

Vine tea is a woody liana plant of Opisthographyaceae. It is widely distributed in Guangdong, Guangxi, Hunan, Hubei and other south regions of the Yangtze River in our country[5]. The Yao, Zhuang and other minorities in China have made tea with its tender leaves after processing and taking it, which has a drinking history of hundreds of years. Vine tea is sweet and cool, with the effect of clearing heat and detoxication, flattening the liver and reducing blood pressure, promoting blood circulation and dredging collaterals. It is mainly used in the treatment of jaundice hepatitis, wind and heat cold, sore throat and other diseases. Modern studies have shown that vine tea has anti-inflammatory analgesic, antibacterial, antioxidant, anti-tumor, liver protection, blood lipid, blood glucose, enhance immunity and other effects[6].

According to the existing literature, a variety of active compounds have been isolated and identified from vine tea, mainly including flavonoids, phenols, steroids and terpenoids, volatile

ISSN: 2688-8645

components, and other chemical components. However, it is still unclear whether vine tea extract can improve the pharmacological effect of HFD-induced fat accumulation. In this study, a mouse model of obesity induced by high-fat diet was established to observe the pharmacological effects of vine tea, and to provide a theoretical reference for the development of its lipid-lowering.

2. Materials and Methods

2.1. Preparation of Vine Tea Extract and Drug Preparation

Vine tea was crushed to obtain the corresponding drug powder and then sieved through a 40-mesh screen. The powder was dried at 60 °C until the weight remained constant and mixed thoroughly before use. Dried and crushed rattan tea (100 g) was placed in a beaker, 1.0 L of ethanol was added, and sonicated three times in a water bath at 40 °C. After ultrasonic extraction, the pooled extracts were concentrated with an evaporator and lyophilized to obtain VTE.

2.2. Animals Administration

SPF male C57BL/6J mice, 22-25 g, were randomly divided into 4 groups after adaptive feeding for 1 week, namely blank control group (normal), model group (HFD), HFD+VTE group (200 and 400mg/kg/), 8 mice in each group. According to the preliminary experiment, VTE was administered by gavage at the doses of 200 and 400mg/kg. The blank control group was fed with normal maintenance diet (25% kcal), and the model group and the VTE administration group were fed with high-fat diet (60% kcal) for 4 weeks. At the end of the 8th week of administration, the mice were anesthetized with pentobarbital, and blood samples were collected from the eyeballs. After the blood was placed at room temperature for 1h, serum was separated by centrifugation at 3000r/min/ and stored at -20 °C for determination of biochemical indicators. At the same time, the liver was harvested and weighed, and the liver index and Lee's index were calculated. One part of the liver was immediately stored in 10% formaldehyde and embedded in paraffin for tissue sectioning. The other part of the liver was stored at -80℃ for frozen section to observe pathological changes.

2.3. Serum Biochemical Indicators

According to the instructions of the kit, the liver function indexes of AST and ALT in serum were detected. The contents of triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) in serum were analyzed.

2.4. Histopathological Testing

Liver tissue fixed in 10% formaldehyde was cut and immersed in running water overnight, dehydrated with conventional gradient alcohol, embedded in paraffin, and made into 5μm paraffin sections. The sections were deparaffinized in xylene, rehydrated in gradient ethanol for H&E staining, dehydrated in gradient ethanol, and the ethanol in the tissue sections was replaced with xylene after dehydration. Neutral resin was dropped and the slides were sealed with a cover glass. The pathological changes of liver tissue sections were observed under a light microscope.

2.5. Statistical Analysis

SPSS 21.0 software was used for statistical analysis. One-way analysis of variance (ANOVA) was used to compare the data between groups. Ridit analysis was used for histological examination comparison, and GraphPad Prism 8.0 software was used for variance analysis and mapping. $P < 0.05$ was considered statistically significant.

ISSN: 2688-8645

3. Results and Analysis

3.1. Effects of VTE on Liver Index, Lee's Index, and Body Weight in HFD-Induced Obese Mice

The effect of VTE on liver indices in mice after 4 weeks of administration is shown in figure 1 Compared with the blank control group, the liver tissue color of the model group was deepened and enlarged, and the liver index was significantly increased ($P < 0.05$). After 4 weeks of administration, both the low and high dose VTE groups could significantly reduce the liver index of mice ($P < 0.05$). Compared with the blank group, the Lee's index of the model group was significantly increased ($P < 0.05$), and the two VTE treatment groups significantly decreased the Lee's index ($P < 0.05$).

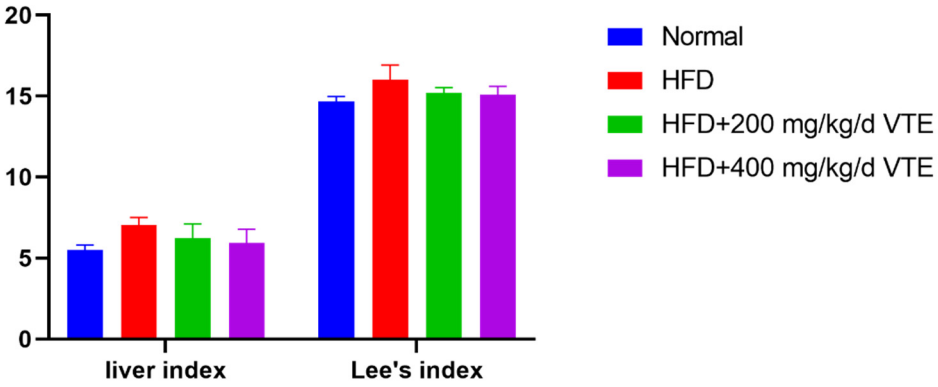

Fig 1. Vine tea extract alleviated the high-fat diet-induced increase in liver index and lee's index

3.2. Effect of VTE on Serum Lipids in Obese Mice

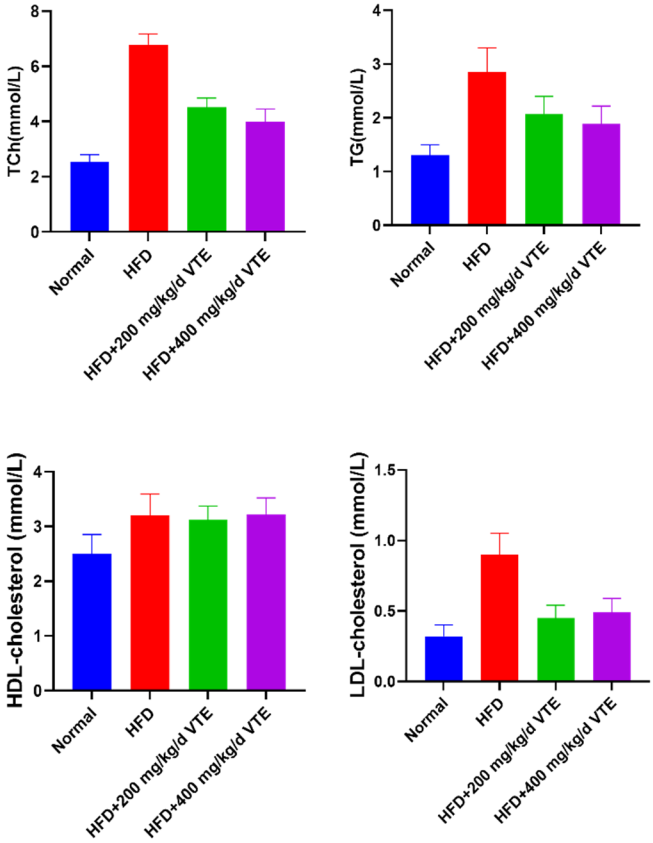

Fig 2. Vine tea extract alleviates hyperlipidemia

ISSN: 2688-8645

Fatty liver and steatosis are often manifested by elevated serum lipids. The changes of serum lipids are mainly related to the amount of body fat content and the use of fat stores, which can reflect the situation of fat metabolism to a large extent. The results are shown in Figure 2 Compared with the blank control group, the levels of TG, TC, LDL-C in serum in the model group were significantly increased, and the level of HDL-C in serum was decreased ($P < 0.05$). Compared with the model group, the levels of TG, TC and LDL-C in serum were decreased, and the level of HDL-C in serum was increased in the VTE low and high dose groups, and there was a certain dose effect relationship ($P < 0.05$).

3.3. Effect of VTE on Liver Function in Obese Mice

AST and ALT are the main enzyme markers of liver injury. When liver cells are injured, these markers will leak from the liver into the blood, resulting in a sharp increase in their serum concentrations. The results are shown in Figure 3 Compared with the blank control group, the serum levels of AST and ALT in the model group were significantly increased ($P < 0.05$), and the activities of AST and ALT in the serum were significantly inhibited in the low and high dose VTE groups ($P < 0.05$). Compared with the model group, the serum levels of AST and ALT in the administration group decreased when the liver was damaged, indicating that VTE has a protective effect on non-alcoholic fatty liver caused by HFD induced obese mice.

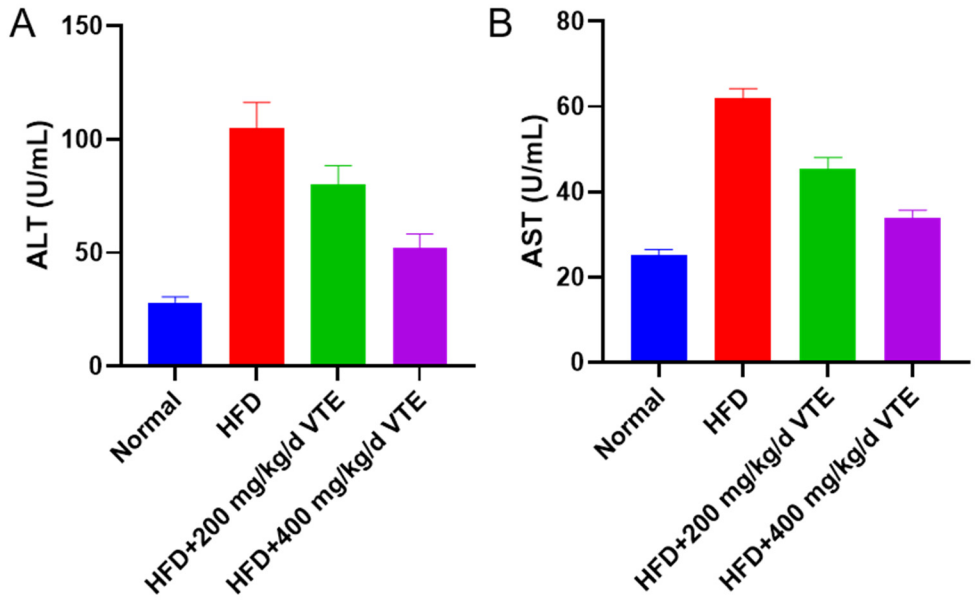

Fig 3. Comparison of serum ALT and AST activities in each group

3.4. Effect of VTE on Liver Pathological Changes in Obese Mice

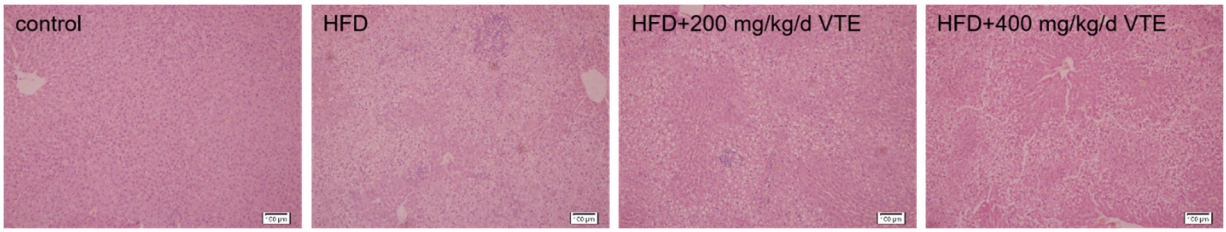

Fig 4. The liver tissue of mice in each group was observed by H&E staining (×100)

In the mice of control group, the structure of liver tissue cells was complete and clear, the structure of liver lobules was normal, the hepatic cords were arranged neatly, and distributed radially around the central vein. The hepatic sinusoids were normal, there was no obvious degeneration of hepatocytes, and the structure of liver nuclei was clear. The liver tissue of the

ISSN: 2688-8645

HFD model group showed diffuse hepatocyte steatosis, mainly manifested as fat vacuoles, accompanied by some cell degeneration and swelling and cell inflammatory infiltration. In the VTE treatment group, fat vacuoles and inflammatory cell infiltration were reduced, and cell necrosis was significantly reduced in the high-dose group, and the arrangement of hepatocytes was more regular. VTE low and high dose groups have certain effects on alleviating steatosis caused by HFD in obese mice. The results are shown in Figure 4.

4. Conclusion

Obesity is one of the common healthy disorders of lipids and carbohydrates, which leads to pathological metabolic disorders such as type 2 diabetes mellitus, hypertension, cardiovascular disease, renal disease and non-alcoholic fatty liver disease. The consumption of high-fat diet is the main risk factor leading to obesity. Long-term intake of high-fat and high-calorie diet will affect the body's normal lipid metabolism, resulting in disorders of fat decomposition, synthesis and storage, leading to abnormal accumulation of fat in the body. Traditional Chinese medicine has unique advantages in the prevention and treatment of non-alcoholic fatty liver disease (NAFLD). Both experimental studies and clinical trials have confirmed that TCM has a good effect in the treatment of NAFLD. The present study investigated the protective effect of VTE against non-alcoholic fatty liver in HFD-induced obese mice. The results of the study showed that the serum levels of AST and ALT were significantly increased in HFD-induced obese mice, indicating that the liver function of the mice was impaired by the high-fat diet, which was improved after the administration of VTE.

In the study, the liver index and Lee's index of the HFD group were found to be increased and statistically significant, which were decreased by VTE treatment. The levels of TG, TC, LDL-C in serum were increased, while the level of HDL-C in serum was decreased in the model group. The levels of TG, TC, LDL-C in serum were decreased, while the levels of HDL-C in serum was increased after VTE treatment. In addition, the results of H&E staining showed that the HFD group had diffuse hepatocyte steatosis, mainly manifested as fat vacuoles, accompanied by some cell degeneration, swelling and inflammatory infiltration, which were significantly improved in the VTE treatment group, indicating that VTE had the effect of alleviating lipid metabolism disorders.

This study demonstrates that VTE has a protective effect on HFD-induced non-alcoholic fatty liver disease in obese mice, and its possible mechanisms include inflammatory response, which provides a theoretical basis for the further research and clinical application of VTE.

References

[1] Jiang, S. Z., Lu, W., Zong, X. F., Ruan, H. Y., & Liu, Y. (2016). Obesity and hypertension. Experimental and therapeutic medicine, 12(4), 2395–2399. https://doi.org/10.3892/etm.2016.3667.

[2] Martin, A., Saunders, D. H., Shenkin, S. D., & Sproule, J. (2014). Lifestyle intervention for improving school achievement in overweight or obese children and adolescents. The Cochrane database of systematic reviews, (3), CD009728. https://doi.org/10.1002/14651858.CD009728.pub2.

[3] Karam J. H. (1992). Type II diabetes and syndrome X. Pathogenesis and glycemic management. Endocrinology and metabolism clinics of North America, 21(2), 329–350.

[4] Di Berardino, C., Peserico, A., Capacchietti, G., Zappacosta, A., Bernabò, N., Russo, V., Mauro, A., El Khatib, M., Gonnella, F., Konstantinidou, F., Stuppia, L., Gatta, V., & Barboni, B. (2022). High-Fat Diet and Female Fertility across Lifespan: A Comparative Lesson from Mammal Models. Nutrients, 14(20), 4341. https://doi.org/10.3390/nu14204341.

[5] Xiao, F., Qiu, J., & Zhao, Y. (2022). Exploring the Potential Toxicological Mechanisms of Vine Tea on the Liver Based on Network Toxicology and Transcriptomics. Frontiers in pharmacology, 13, 855926. https://doi.org/10.3389/fphar.2022.855926.

ISSN: 2688-8645

[6] Carneiro, R. C. V., Wang, H., Duncan, S. E., & O'Keefe, S. F. (2020). Flavor compounds in Vine Tea (Ampelopsis grossedentata) infusions. Food science & nutrition, 8(8), 4505–4511. https:// doi.org/ 10. 1002/fsn3.1754.

[7] Shao, Q., Wu, Y., Ji, J., Xu, T., Yu, Q., Ma, C., Liao, X., Cheng, F., & Wang, X. (2021). Interaction Mechanisms Between Major Depressive Disorder and Non-alcoholic Fatty Liver Disease. Frontiers in psychiatry, 12, 711835. https://doi.org/10.3389/fpsyt.2021.711835.

[8] Zhang, S., Wong, Y. T., Tang, K. Y., Kwan, H. Y., & Su, T. (2020). Chinese Medicinal Herbs Targeting the Gut-Liver Axis and Adipose Tissue-Liver Axis for Non-Alcoholic Fatty Liver Disease Treatments: The Ancient Wisdom and Modern Science. Frontiers in endocrinology, 11, 572729. https:// doi.org/ 10. 3389/fendo.2020.572729.