Effects of Enshi Selenium-Rich Tea Combined with Gynostemma Pentaphyllum and Apenma on Lipid Reduction in Hyperlipemia Mice

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Research

Abstract

Objective: To investigate the effects of tea blend composed of Enshi selenium-rich tea, gynostemma pentaphyllum and apenma on blood lipid and lipid metabolism in mice with hyperlipidemia and prevention of hyperlipidemia in mice on the high-fat diet.

Methods: Mice were randomized into 7 groups, among which 6 groups were fed with high-fat diet to establish a hyperlipidemia mouse model, and the other group was fed with normal diet as the normal control. After the hyperlipidemia model was established, mice were fed with normal diet while receiving different regimens. The normal diet group, the high-fat control group and the positive control group were given physiological saline solution, physiological saline solution and atorvastatin daily by gavage, respectively. The initial intervention group was given medium dose tea blend solution by gavage, and the other three groups were given low, medium and high dose of tea solution daily by gavage, respectively. After 4 weeks of treatment, mice were sacrificed, blood samples were taken for monitoring of lipid metabolism, -liver and tissue tissues were removed for examination of morphology and gene expression.

Results: The tea blend not only significantly reduced the level of blood lipid in hyperlipidemia mice, but also effectively protected the liver and kidney function ($P < 0.05$). Observation under the light microscope reveled that, high-fat diet led to the accumulation of fat in hepatocytes and disorder of hepatic cordage, while the tea blend alleviated the hepatocyte steatosis. In addition, the tea blend promoted the expression of HL and HSL genes, and increased the levels of LAXR and PPARA, which regulated lipid metabolism at the genetic level ($P < 0.05$).

Conclusion: This study confirmed that drinking tea blend composed of Enshi selenium-enriched tea, Gynostemma pentaphyllum and Apocynum venetum could lower blood lipids in mice with hyperlipidemia. In addition, various doses of tea blend could be used for hyperlipidemia with good liver and kidney function, and medium or high dose of tea blend was relatively safe for hyperlipidemia with poor liver and kidney function.

1. Introduction

Hyperlipidemia is an increase in cholesterol, triglycerides, and low-density lipoprotein cholesterol. Dyslipidemia is an independent risk factor for cardiovascular disease. The risk of cardiovascular disease in patients with hyperlipidemia is twice that of normal people, which is a serious threat to human health. LDL-C is directly involved in the formation of subcutaneous lipid plaque and is closely related to the process of atherosclerosis.[1] Diets rich in saturated fatty acids have a significant impact on plasma lipid levels, which are closely related to liver injury and can induce the development of metabolic disorders, coronary heart disease, non-alcoholic fatty liver disease and other diseases. This is because the accumulation of triglycerides (TG) can induce non-progressive sclerosis, while the accumulation of cholesterol can cause the hepatocytes to swell.[2, 3] In addition, cholesterol, as a special member of the lipid family, plays an important role in biofilm synthesis and cell proliferation and differentiation.[4]

With changes in habits and lifestyle, the incidence of hyperlipidemia increased every year. According to statistics, the prevalence of hyperlipidemia was about 13.1% in China, 9.2% in South Korea and 6.9% in Japan.[5] A national health and nutrition examination survey in the United States showed that 11.7% of adults aged 20–39 and 41.2% of adults aged 40–64 had elevated low-density lipoprotein cholesterol (LDL-C) levels. [6]

Enshi selenium-enriched tea, produced in Enshi City, Hubei Province, China, has a selenium content of 0.2–0.4 mg/kg. In recent years, research on selenium-enriched tea has shown that selenium-enriched tea can improve the metabolism of blood lipids and lipoproteins in mice on a high-cholesterol diet.

Gynostemma pentaphyllum is a perennial herbaceous vine of Gynostemma pentaphyllum belonging to Cucurbitaceae, which is rich in total brass, saponin and various trace elements. Gynostemma pentaphyllum plays an anti-hyperlipidemia role by increasing the level of phosphatidylcholine and reducing the level of trimethylamine N-oxide (TMAO), and is widely used in the treatment of hyperlipidemia in China.[7, 8] Gynostemma pentaphyllum has also shown anti-inflammatory effects in some studies.

Apocynum is the dried leaves of Apocynum in Oleander family. The flavonoids in Apocynum have functions such as lowering blood lipid, protecting cardiovascular endothelium and protecting liver.[9] However, some studies have found that apocynum has cardiotoxicity.

The purpose of this study was to investigate whether the tea blend made from Enshi Selenium-enriched Tea, Gynostemma pentaphyllum and Apocynma had better effects of lowering blood lipid and anti-inflammatory, as well as whether there was hepatorenal toxicity.

2 Materials And Methods

2.1 Subjects

2.1.1 Experimental animals

A total of 70 5-week-old C57BL/6J mice (SPF grade) were purchased from Beijing Vitong Lihua Experimental Animal Technology Co., Ltd. The animal quality certificate number was SCXK (Beijing) 2016-0006, and the license number of the experimental unit was SYXK (Hubei) 2016-0057.This study was reviewed and approved by the Ethics Committee of Huazhong University of Science and Technology.

2.1.2 Drugs and reagents

In this study, the intervention drugs were Hubei Enshi Selenium-enriched Tea (provided by Xianfeng Tangya Tea Functional Research Center, Hubei, China), Gynostemma pentaphylum, Atorvastatin and physiological saline.

The kits used in this experiment were as follows: triglyceride TG, high-density lipoprotein cholesterol HDL-C, total cholesterol T-CHO, alanine aminotransferase ALT, aspartate aminotransferase AST, and low-density lipoprotein cholesterol LDL-C (Changchun Huili); Mouse IFN-γ (Interferon Gamma) ELISA Kit (Item No.: E-El-M0048C Elarite); Mouse IL-6(Interleukin 6) ELISA Kit (E-EL-M0044C Elerite); Mouse TNF-α (Tumor Necrosis Factor Alpha), ELISA Kit (E-EL-M0049C).

2.1.3 Formula of high fat feed

The main components and contents of the high-fat feed used in this study (Catalogue No.: D09100301, Wuhan Shanghe Biological Technology Co., Ltd.) are as follows: Protein 22.5 gm%, carbohydrate 44.9 gm%, fat 19.9 gm%, casein 200 gm%, cystine 3 gm%, maltodextrin 100 gm%, fructose 200 gm%, sucrose 96 gm%.

2.1.4 Main experimental instrument

The main experimental instruments used in this experiment are as follows: FlexStation 3 Multifunctional Molecular Devices, Micro High Speed Centrifuge (C2500-R-230V Labnet), Electronic Balance (Beijing Sateris Instrument System Co., Ltd. CPA),ELISA (Thermo MµLISKMK3), real-time quantitative PCR (ABI Quant Studio 6), horizontal electrophoresis (Beijing Junyi Oriental Electrophoresis Equipment Co., Ltd. JY300), ultraviolet analyzer (Beijing Junyi Oriental Electrophoresis Equipment Co., Ltd. JY02S),Pathological slicer (Germany Leica RM 2016 rotary slicer), dehydrator (Wuhan Junjie JT-12J computer biological tissue dehydrator), embedding machine (Wuhan Junjie JB-P5).

2.2 Experimental method

2.2.1 Preparation of tea solution

Based on the maximum dose of 10g/day dry tea for adults and the ratio table of body surface area between experimental animals and human, the daily dose of dry tea for mice was calculated. The tea blend was prepared from Enshi Selenium-rich tea, Gynopentaphylum pentaphylum and apocynum in the proportion of 10:2:0.5. The tea was soaked in boiling water at about 95°C for 6 minutes with even stirring. The container was let stand still until solid components settled. The supernatant was then removed, filtered and stored in a sterile glass bottle in a refrigerator at 4°C until use. In order to maintain consistency in quality, the tea solution was prepared once a week. The doses of tea blend were 0.75, 1.5 and 2.25g dry tea/kg weight for the low, medium and high dose group respectively. Atorvastatin was given at a dose of 10mg/kg and dispensed as needed.

2.2.2 Mice bred

The model of hyperlipidemia was established after an adaptive feeding of normal feed for 2 weeks followed by a high-fat feed for 8 weeks. Finally, it was changed to normal feed for 4 weeks.

2.2.3 Animal grouping, modeling, and intervention methods

Seventy mice were randomly divided into 7 groups with half males and half females in each group: the normal control group (group A), the hyperlipidemia control group (group B), the positive control group (group C), the initial tea group (group D). the low dose tea group (group E), the medium dose tea group (group F), and the high dose tea group (group G). At the beginning of the study, group A was fed with normal diet, and group B-G was fed with high-fat diet. Group D was given medium dose of tea blend solution by gavage at the beginning of the experiment. After 8 weeks, groups A-G were given physiological saline, physiological saline, atorvastatin, medium dose tea blend, low dose tea, medium dose tea, and high dose tea, respectively, by gavage at a volume of 0.1ml/10g body weight, once a day. At the same time, they were fed with normal diet for 4 weeks.

2.2.4 Specimen collection and processing

In the end of the study, all mice were water fasted for 24 hours. After intraperitoneal injection of anesthesia, blood was collected from the venous sinus. The blood sample was centrifuged at 3000 rpm for 10min, and supernatant collected for laboratory examinations. The mice were sacrificed by cervical dislocation, and the abdominal cavity and thorax were exposed. Liver, kidney, heart, and pancreas tissues were removed, rinsed with physiological saline, weighed and fixed in 4% formaldehyde solution.

2.2.5 Detection index and method

2.2.5.1 Blood biochemical index detection

Blood biochemical indexes including triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and creatinine (CR), were determined by using an automatic biochemical analyzer (Chemray240, Rayto).

2.2.5.2

Levels of inflammatory cytokines (IL-6, TNF-α, IFN-γ) were determined by ELISA kit.

2.2.5.3 H&E staining

Fixed tissues were subjected to, dehydration, clearing and paraffin embedding by the automatic procedure of a dehydrator, and sectioning was performed with a Leica pathological slicer. The paraffin slices were rehydrated and stained with Mayer's hematoxylin dye for 5min. The slices were then rinsed with tap water and stained with 1% water-soluble eosin dye solution for 5 minutes before being rinsed with tap water for 30 seconds. The slices were then treated with 5% ethanol for 30 seconds, 95% ethanol for 1 minute, 100% ethanol I for 5 minutes x 2, xylene for 5 minutes x 2, air dried and examined under a microscope.

2.2.5.4 qRT-PCR:

Total RNA was extracted using the Trizol reagent, and cDNA was made by transcription. Gene expression was determined on a real-time quantitative PCR instrument (ABI Quantstudio 6). Primer sequences for individual genes are shown in Table 1. The mRNA levels of all genes were normalized against house-keeping gene GAPDH.

Table 1
Primer sequences for qRT-PCR.

Gene	Primer	Sequence (5'-3')	PCR Products
Mus GAPDH	Forward	ATGGGTGTGAACCACGAGA	229bp
	Reverse	CAGGGATGATGTTCTGGGCA	
Mus HSL	Forward	GACTCACCGCTGACTTCC	161bp
	Reverse	TGTCTCGTTGCGTTTGTAG	
Mus HL	Forward	CCCACTATGACTTCTACCCC	298bp
	Reverse	CTCTTGCTCTTGCCTGACC	

2.2.5.5 Western blot analysis

Liver tissue was fully lysed to extract total proteins from the tissue. The protein content in the sample was determined by using the UV assay. Samples with sampling dye were boiled for 10 minutes of deformation and resolved on PAGE gels by electrophoresis. The resolved proteins were then transferred to PVDF membranes. The PVDF membrane was blocked with at room temperature for 2h. The membrane was incubated with the primary antibody overnight at 4°C. The membrane was rinsed three times with TBST buffer and incubated with the secondary antibody with shaking at room temperature for 2h. The membrane was rinsed with TBST buffer x3 to wash off the secondary antibody. The membrane was then treated with The ECL Western Blotting Substrate and analyzed with BandScan.

2.2.6 Statistical analysis

Continuous variables are expressed as mean ± standard deviation. Homogeneity test of variance, t test and one-way analysis of variance were used to compare the means of multiple groups. All statistical analyses were performed using SPSS version 22(Chicago, IL, USA), and $P<0.05$ was considered statistically significant. GraphPad Prism 8.0 software was used to draw the histogram and line chart.

3 Results And Analysis

3.1 Body weight and tissue weight of mice

Statistical analyses showed, compared with group A, there were no significant differences in body weight and tissue weight of heart, kidney and thymus of mice in group B, but liver weight of mice in group B was

significantly higher than that in group A, indicating that the process of establishing hyperlipidemia model may cause fatty liver. There were no significant differences in body weight and tissue weight of heart, kidney, thymus and other tissues between C-G group and B group, but liver weight of mice in C-G group was lighter than that in B group, manifesting that atorvastatin and tea blend may reduce fatty liver. (Fig. 1)

3.2 Reducing blood lipid levels

The levels of TC, TG and LDL-C in group A were lower than that of group B, showing that the hyperlipidemia model was successfully established by feeding high fat diet($P<0.05$). The levels of TC, TG and LDL-C in group C were significantly lower than that in group B, while the HDL-C level of group C was higher than that of group B, demonstrating that atorvastatin can significantly reduce the level of blood lipid in hyperlipidemia mice($P<0.05$). The levels of TC, TG and LDL-C in group D were significantly lower than that in group B, while the HDL-C level in group D was higher than that of group B. It suggested that early tea administration can significantly inhibit the increase of TC and TG, maintain low levels of TC and TG, and high level of HDL, and inhibit the increase of LDL level ($P<0.05$). Compared with group B, levels of TC, TG and LDL-C in group E, F and G were decreased while HDL-C was increased, revealing that tea blend could significantly reduce the level of blood lipid at all different doses tested ($P<0.05$) (Fig. 2).

3.3 Improving liver and kidney function

This study found that the levels of AST, ALT and CR in group B were notably higher than that in group A, suggesting that hyperlipidemia may cause damage in liver and kidney($P<0.05$). AST, ALT and Cr levels in group C and group G were significantly lower than those in group B, demonstrating that atorvastatin and high dose tea blend can effectively reverse functional damage as a result of hyperlipidemia in liver and kidney tissues ($P<0.05$) (Fig. 3).

3.4 Reducing inflammation and inhibit the release of inflammatory factors

The levels of TNF-α, IL-6 and IFN-γ in group B were significantly lower than those in group A, indicating that high-fat diet can cause inflammatory response and promote the release of inflammatory factors ($P<0.05$). Compared to group B, the levels of TNF-α, IL-6 and IFN-γ in group C and group G were lower, suggesting that atorvastatin and high dose tea blend reduced the release of inflammatory factors induced by hyperlipidemia and alleviated the inflammatory response ($P<0.05$) (Fig. 4).

3.5 Improving hepatocyte steatosis

To investigate whether the tea blend could affect hepatocyte morphology, we performed pathological analysis on mice liver cells. HE staining, showed that hepatocyte steatosis was more common and hepatic cord arrangement was more disordered in group B than in group A. Compared with group B, C-G

group showed less steatosis and less disorder of hepatic cord arrangement. These results suggest that atorvastatin and tea blend could improve hepatocyte steatosis (Fig. 5a, b).

3.6 Promoting HL and HSL gene expression

In order to further explore the mechanism how the tea blend lowered blood lipid, RT-PCR was performed to detect mRNA levels of HL and HSL in mice. qRT-PCR analyses showed, compared with group A, HL mRNA level was significantly lower in group B, demonstrating that high-fat diet could reduce the expression level of hepatic lipase gene (P<0.05). The level of HL mRNA in groups C, D, F and G was significantly higher than that in group B, showing that atorvastatin and the tea blend could reverse the decrease of HL expression caused by high fat diet (P<0.05). Besides, HSL mRNA level was significantly lower in group B than in group A, suggesting that high-fat diet reduced the expression of hormone-sensitive lipase (P<0.05). HSL mRNA level in C-G group marginally higher than that in B group (P>0.05) (Fig. 6). Both the tea blend and atorvastatin could reverse the decreased levels of hepatic lipase and HSL in the short term, although the difference was not constant statistically significant. The effect was related to the tea blend concentration and the intervention mode. The effect of initial tea administration and medium and high dose tea administration were related to those of atorvastatin.

3.7 Promoting the expression of LAXR and PPARA

To verify the above results, western blot analysis was performed. Western-blotting showed that, compared with group A, the level of LAXR in group B was significantly lower, indicating that hyperlipidemia could lead to significant decrease in LAXR expression (P<0.05). The level of LAXR in group C was significantly higher than that in group B, suggesting that atorvastatin can reverse the decrease of LAXR expression caused by hyperlipidemia (P<0.05). The level of LAXR in group D, F and G was significantly higher than that in group B, suggesting that medium and high doses of tea blend could also reverse the decrease of LAXR expression caused by hyperlipidemia (P<0.05). In fact, early administration of the tea blend increased the LXRs level to the greatest degree among all groups. In addition, PPARA in group B was significantly lower than that in group A, demonstrating that hyperlipidemia could lead to significant decrease in the expression of PPARA (P<0.05). PPARA in group B was significantly lower than that in group C-G, representing that atorvastatin and different doses of the tea blend could effectively reverse the reduction of PPARA expression level caused by hyperlipidemia (P<0.05) (Fig. 7, 8).

4. Discusion

The results of this study showed that tea blend composed of Enshi-Selenium-enriched tea, Gynostemma pentaphyllum and Apenema could dose-dependently reduce the level of blood lipid. The effect of different concentrations of tea blend on lowering blood lipid was close to that of atorvastatin. Furthermore, drinking the tea blend early could effectively prevent the occurance of hyperlipidemia. The possible reasons for the reduction of blood lipid in tea blend are as follows. On the one hand, tea polyphenols in tea leaves could bind with lipids after forming oxidized tea polyphenols, reduce lipid

deposition in liver and visceral white adipose tissue, thereby improving lipid metabolism to a large extent.[10] Other studies have shown that tea polyphenols could regulate blood lipid levels by improving the gut microbiome associated with a high-fat diet.[11] On the other hand, Gynostemma pentophyllum could increase lipid absorption and reduce lipid deposition in blood vessel walls by inhibiting TMAO pathway. In addition, Apenema leaves affect lipid metabolism by acting on AKT1 pathway and MAPK pathway.

The reason why the tea blend reversed the damage to liver and kidney function may reside in that epigallocatechin-3-gallate (EGCG) can reduce the reabsorption of bile acids and reduce the absorption of lipids. Meanwhile, EGCG also has a protective effect on kidney function due to its antioxidant and anti-inflammatory properties. [12–14] Low dose tea caused greater damage to liver and kidney function, possibly because its antioxidant and anti-inflammatory effects were not enough to offset the burden on liver and kidney metabolism.

This study also demonstrated that administration of the tea blend, at different doses tested or with different timing, could reduce the levels of TNF-α, IFN-γ, IL-6 and other inflammatory factors, especially when administered from an early time or at a high dose. In fact, whether given early or in high doses, the effect was similar to that of atorvastatin in inhibiting inflammatory cytokine release. This is consistent with previous reports showing that tea polyphenols could inhibit the signal transduction of Toll-like receptor 4 (TLR4) pathway, and thus reduced the levels of inflammatory factors such as TNF-α and IL-6.[14, 15]

When the average body weight and tissue weight of mice in each group were compared between different groups, it was found that the body weight and tissue weight of mice given the tea solution were not significantly different from those of the control group. Neither tea nor atorvastatin had significant effects on the body weight, liver and kidney tissue weight of mice. However, difference might emerge if more prolonged treatment were given to the animals.

With HE staining, it was observed under the light microscope that the tea blend could reduce the damage of hepatocytes in metabolism-related fatty liver disease and improve the disordered hepatic cord structure caused by lipids. Our data strongly suggested that early administration of the tea blend could efficiently prevent hepatocyte adipogenesis.

Previous studies have shown that HL mainly decomposes TG in CM, HDL and MDL, and participates in the formation of LDL and the cholesterol anti-transport process. HSL is the rate-limiting enzyme of lipolysis, which is involved in the decomposition of triglycerides and diglycerides (DG) to generate free fatty acids (FFA), regulating fatty acid β oxidation, which provide energy for the body. [16] Both HL and HSL play critical roles in lipid metabolism, overall energy balance, and cellular events that may involve fatty acid signaling.[17–19] The results of this study showed that the mechanism of tea blend lowering blood lipid may be closely related to maintaining high levels of HL and HSL.

The results showed that the tea and atorvastatin also increased LXRs levels in hyperlipidemic mice. However, compared with high-fat control group, low dose tea could not effectively increase the level of LXRs. Studies have shown that the activation of LXRs can promote the reverse transport of cholesterol, increase the transcription of rate-limiting enzymes in the process of bile acid synthesis in the liver, [20] accelerate the degradation of low-density lipoprotein receptor(LDLR) and thereby reduce cholesterol uptake in liver cells.[21] PPARα is expressed primarily in hepatocytes and is a transcriptional activator of fibroblast growth factors 21, FGF21, ACOX1 and ATF6. It is a key regulator of glucose and lipid metabolism by regulating ketogenesis, gluconeogenesis and lipolysis in liver. The results of this study showed that compared with the hyperlipidemia control group, both the tea blend and atorvastatin could significantly increase PPARα level, and the effect of early tea administration had the strongest effect on PPARα level. PPARα may reduce lipid synthesis by inhibiting HMG-CoA reductase, and this effect may be synergistic with LXRs. Thus, the tea blend can regulate lipid metabolism by regulating the expression of PPARα and LXRs. [22]

In summary, this study confirmed that drinking the tea blend composed of Enshi Selenium-rich tea, Gynostemma pentaphyllum and Apocynma significantly reduced blood lipid in mice with hyperlipidemia, and was safe. For hyperlipidemia patients with good liver and kidney function, various dose of tea blend is appropriate to drink. While for hyperlipidemia patients with liver and kidney dysfunction, it is recommended to drink medium or high dose tea blend. For people who are used to high-fat diet, early start administration of a medium dose of tea blend, can effectively prevent the onset of dietary hyperlipidemia.

The limitations of this study are as follows: on the one hand, this study is a cross-sectional study, so there may be some limitations in the results, and the conclusions need to be verified by further longitudinal studies. On the other hand, this study has not gone deep into the molecular mechanism, and further experiments are needed to explore the mechanism of tea blend lowering blood lipid.

Conclusion

Our study found that the tea blend made by enshi selenia-rich tea, gypentaphyllum gypentaphyllum and apocynum apocynum in proportion can effectively prevent and treat hyperlipemia and reverse the damage of liver and kidney function.

Declarations

Ethics approval and consent to participate

This research was reviewed and approved by the Ethics Committee of Huazhong University of Science and Technology.

Consent for publication

Not applicable.

Availability of data and material

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

Competing interests

The authors have no financial or non-financial competing interests to declare.

Funding

Not applicable.

Authors' contributions

Xiaoling Liu and Ze Zhang conducted experiments and analyzed experimental data. Yan Qin processed tissue samples and interpreted pathological findings. Fang Zou, Gui Wang, Guilin Xing, and Zhong Huang were mainly responsible for the formulation of experimental intervention drugs. Jun Hu and Bende Liu designed the experimental scheme and analyzed the final experimental results.

Acknowledgements

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Figures
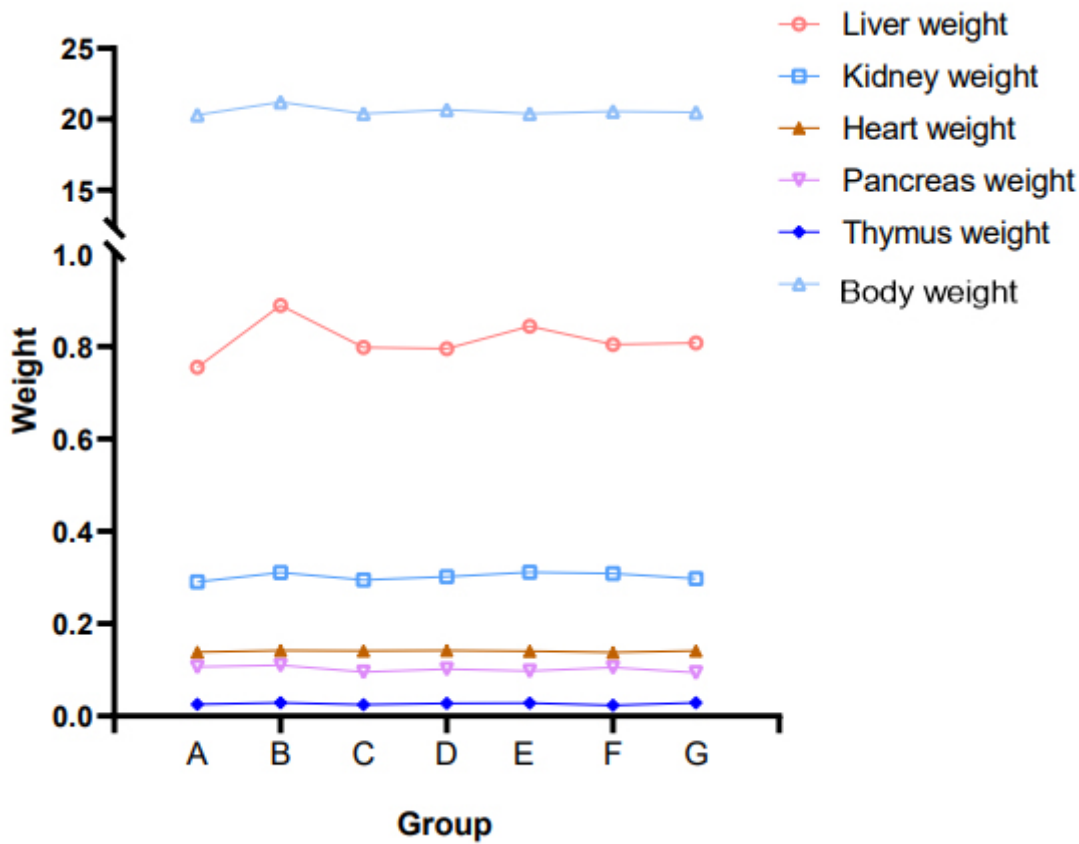
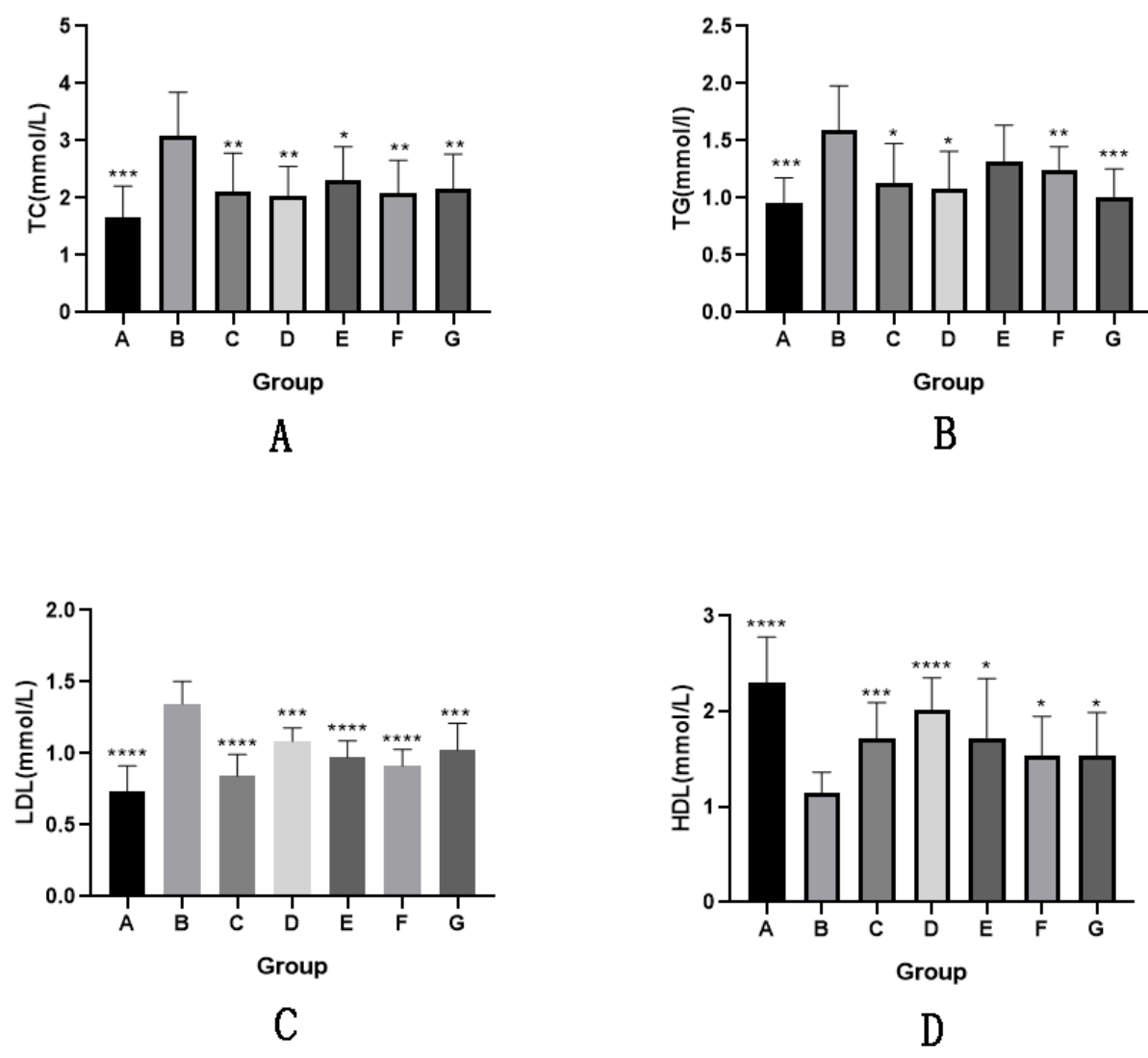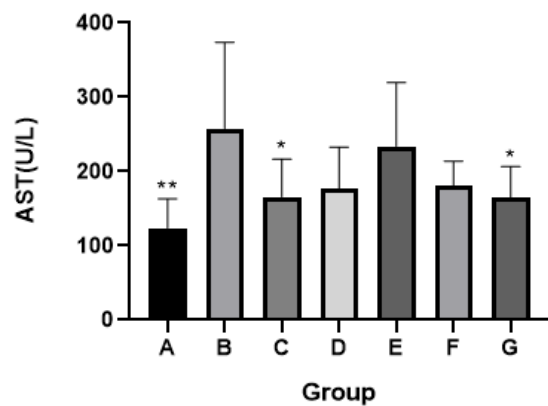**Figure 1**

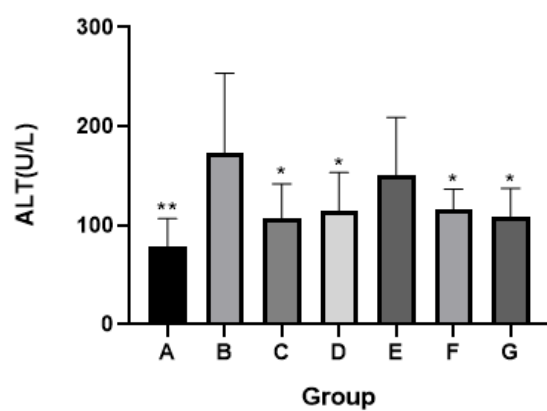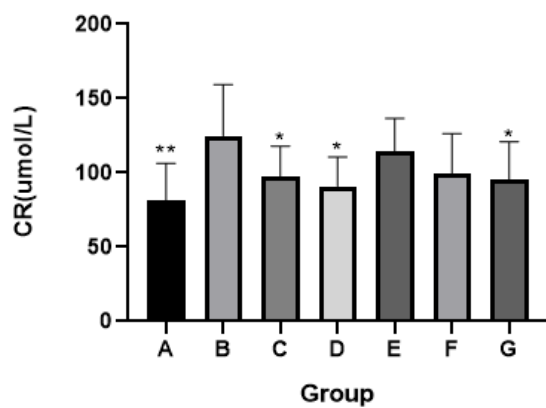Body weight and tissue weight of mice in each group.

Figure 2

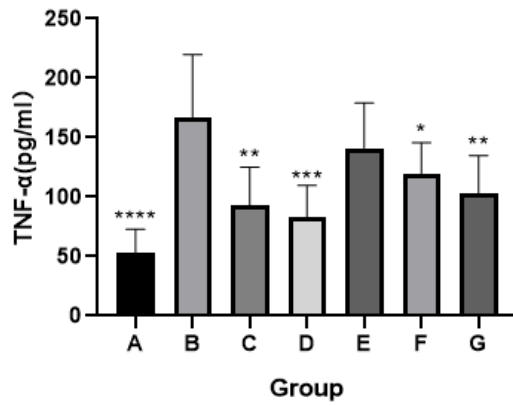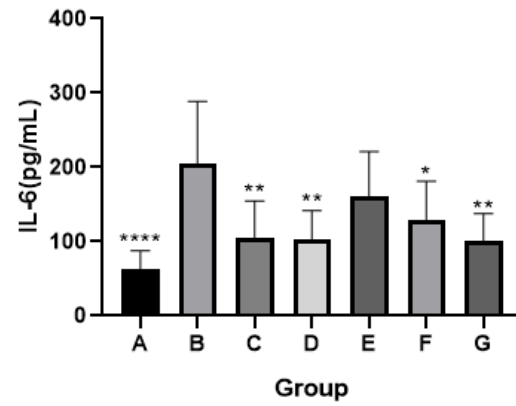The blood lipid level of mice in each group.
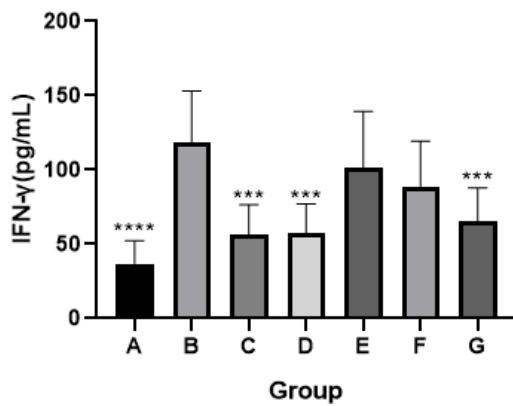
A

B

C

Figure 3
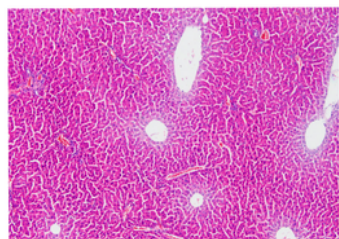
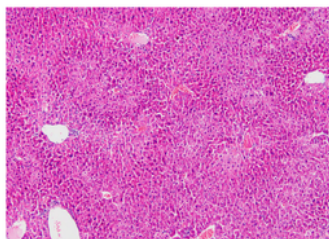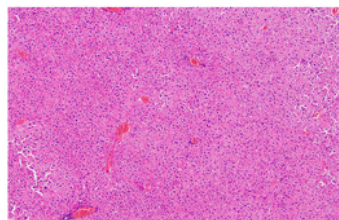Liver and kidney function indexes of mice in each group.

Figure 4

The levels of inflammatory cytokines in each group.
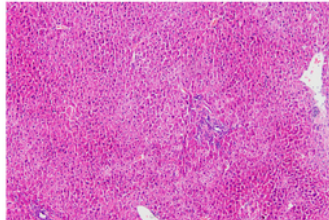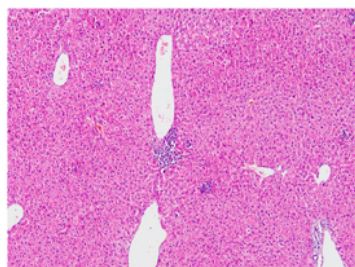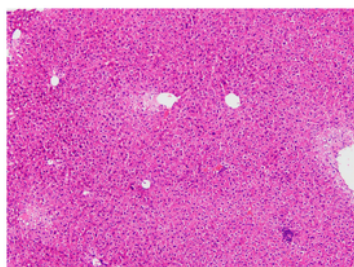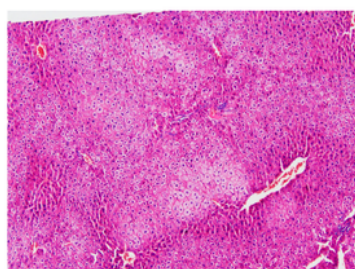
Figure 5

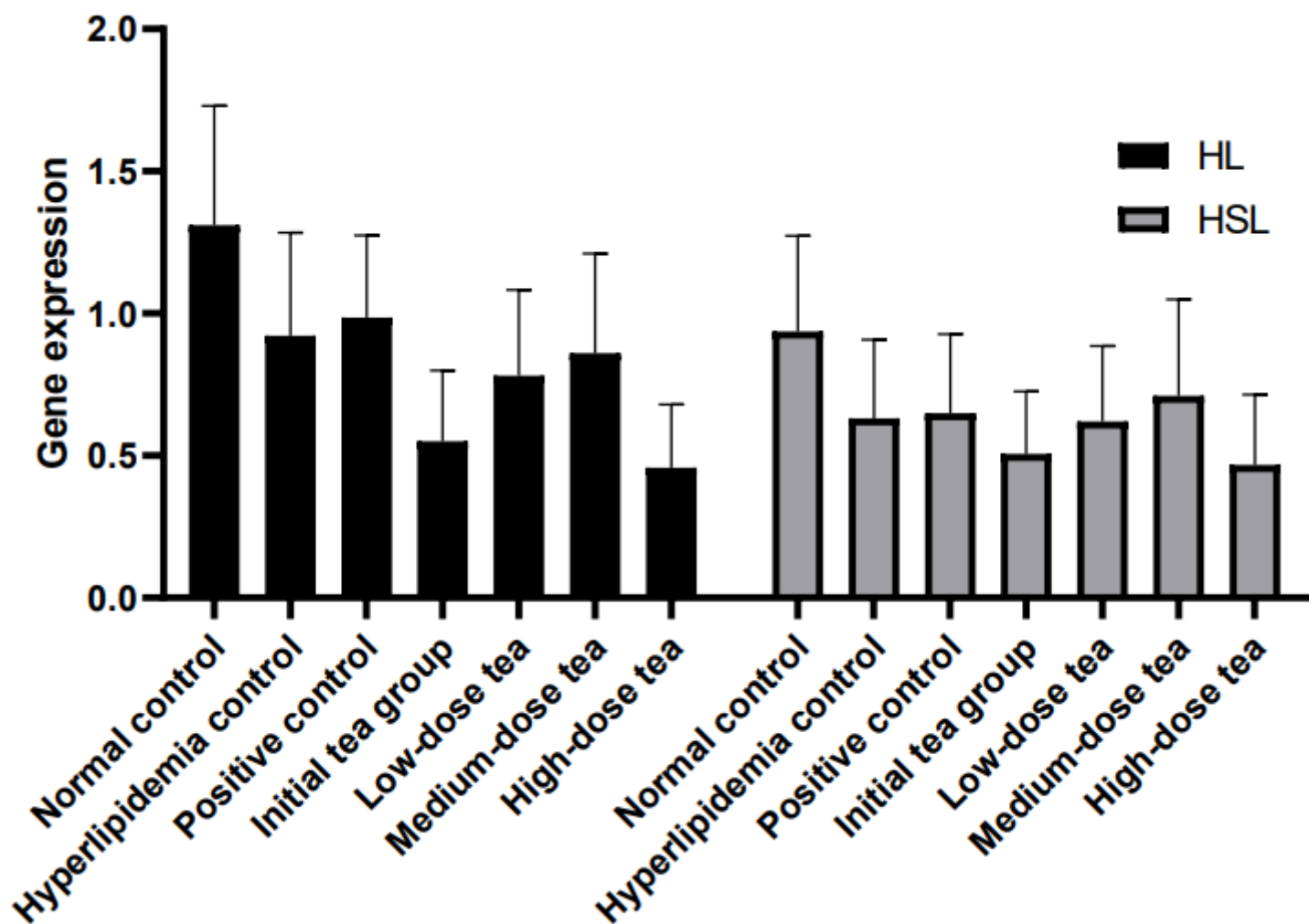Typical pathological images of hepatocytes of mice in each group under HE staining.

Figure 6
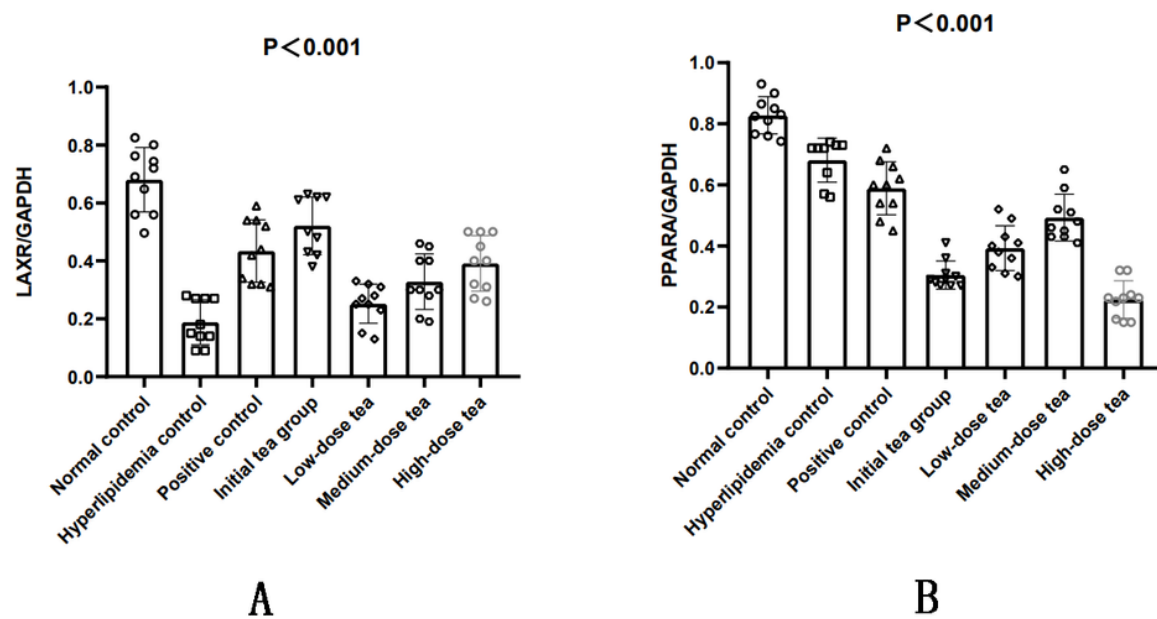
Expression levels of HL and HSL in mice in each group.

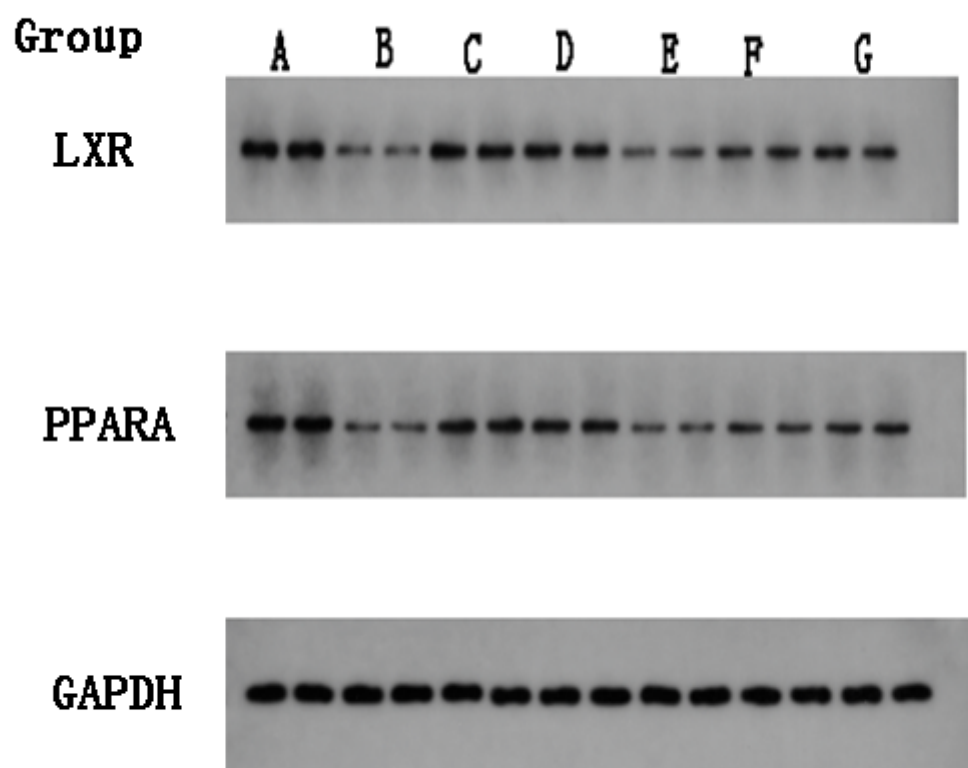**Figure 7**

LXR and PPARA levels in each group.

Figure 8

Image of gel electrophoresis for Western blot analysis.