Hypolipidemic effect of *Fragarianilgerrensis* Schlecht. medicine compound on hyperlipidemic rats

Liangcai Gao *, Zejie Lin †, Yilian Liu †, Xinyi Wang, Linlin Wan, Liuliu Zhang and Xinnan Liu

**Abstract**

**Background:** *Fragarianilgerrensis* Schlecht. medicine compound (FN-MC) is a kind of Chinese herbs' compound consisted of *Fragarianilgerrensis* Schlecht. and *Centella asiatica* (L.) Urban. The study was to investigate the hypolipidemia effect of FN-MC in a hypolipidemic rat model.

**Methods:** Male SD rats were randomly divided into five groups: normal-fat diet (NFD) group, high-fat diet (HFD) group, FN-MC (2 g/Kg) group, FN-MC (4 g/Kg) group and simvastatin (PDC) group. After FN-MC treatment, body weight, food intake, serum and hepatic biochemistry parameters of rats were measured and the pathological changes of liver and its cells were observed by optical microscope and transmission electron microscopy.

**Results:** The results showed that FN-MC significantly decreased the levels of serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL-C), apolipoprotein B (ApoB) and hepatic malondialdehyde (MDA), while increased serum high-density lipoprotein (HDL-C), apolipoprotein A1 (ApoA1) and hepatic Superoxid e Dismutase (SOD). FN-MC also improved the structure of liver and decreased the lipid drops in the cytoplasm significantly. In addition, FN-MC significantly decreased the weight gain and had no significant effects on food intake.

**Conclusions:** The study suggested that FN-MC exhibited strong ability to improve the dyslipidemia and prevent hepatic fatty deposition in rats fed with high-fat diet. Meanwhile, FN-MC exerted anti-obesity and antioxidant properties.

**Highlights:**

- *Fragarianilgerrensis* Schlecht. medicine compound possesses a hypolipidemic effect on hyperlipidemic rat model
- *Fragarianilgerrensis* Schlecht. medicine compound administration improves the antioxidant capacity of rats
- *Fragarianilgerrensis* Schlecht. medicine compound prevents hepatic fatty deposition

**Keywords:** *Fragarianilgerrensis* Schlecht., *Centella asiatica* (L.) urban., Hyperlipidemia, Antioxidation, Histopathology

**Background**

Hyperlipidemia is a kind of metabolic disorder disease which involves an abnormally high level of blood lipids and lipoproteins. It is a major cause of arteriosclerosis, cerebral stroke, coronary heart disease, myocardial infarction and renal failure in Chinese people [1–3]. At present, the main treatment of hyperlipidemia is exercise, smoking cessation, dietary therapy and medication. Statins, nicotinic acids and bile acid sequestrants are the most commonly used medicine by far which can reduce lipids and lipoproteins in the blood. It has been shown that those drugs are beneficial in patients with preventing many kinds of cardiovascular disease [4]. Although they are effective in modulating hyperlipidemia in both preclinical and clinical studies, their toxicity of liver and kidney can’t be ignored, so a lot of researchers are working on finding more effective drugs to put into treatment [5–7]. In recent years, Chinese medicine has received great
attention of many scholars for its stabilizing effect and few side-effects [6, 7].

*Fragarianilgerrensis* Schlecht. is a plant which belongs to Rosaceae, Fragaria. It grows at grasslands of mountain slope or forests on river bank which are at an altitude of 700-3000 m and it mainly distributes in Yunnan, Guangxi of China. *Fragarianilgerrensis* Schlecht has perfect effect of heat-clearing and detoxifying and it can activate blood circulation to dissipate blood stasis. People in Yunnan, China usually make it into mixture with *Centella asiatica* (L.) Urban [8, 9] and use the mixture as a folk remedy to treat cardiovascular diseases.

In our experiment, we fed the rats with high-fat diet to establish hyperlipidemic rat model. Then we divided the rats into NFD group, HFD group, FN-MC (2 g/Kg) group, FN-MC (4 g/Kg) group and PDC group. After the drug treatment, total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) in serum were measured to evaluate the hypolipidemic effect of FN-MC [10–12]. Meanwhile, we used optical microscope and electron microscopy to observe the changes of morphology and ultrastructure of liver tissue. In addition, we measured the activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in the liver homogenate to study the changes of rats’ antioxidant capacity [13–15].

**Methods**

**Drug preparation**

The whole plants of *Fragarianilgerrensis* Schlecht. and *Centella asiatica* (L.) Urban (including roots, stems and leaves) were cut up after drying. Each herbal medicine weighed 12 g, and leaching by 500 ml of 75% ethanol at room temperature for 3 h. Cooked and concentrated the compound under vacuum in a rotary evaporator at 40 ± 5 °C for 1.5 h and then filtrate to obtain the extract to use.

The dose of simvastatin for human is 20 mg per day. The dose for rat was calculated by the human equivalent dose (HED) on the basis of body surface area: assuming a human weight of 60 kg, the HED for 20 (mg)/60 (kg) = 1/3 mg/kg [16]; rat dose converted by body surface area was 2 mg/kg; the experimental effective dose was twice as high as the clinical dose, so it was 4 mg/kg. Each simvastatin capsule was dissolved in 50 ml of water to form a simvastatin solution at a concentration of 0.4 g · ml⁻¹ [16].

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**Experimental animals**

A total of 30 male Sprague-Dawley rats (180 ± 20 g body weight) were obtained from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). The rats were housed at 22 ± 2 °C with free access to food and water, under a 12:12 h light/dark cycle (lights on at 08:00 h). All experimental methods were approved by the Institutional Review Boards of East China Normal University, and they were performed in accordance with relative guidelines and regulations.

**Animal grouping**

After acclimation for two weeks, the rats were randomly divided into two groups: normal-fat diet (NFD) group (n = 6) and high-fat diet (HFD) group (n = 24). Rats of HFD group were fed with high-fat fodder of 60% calorie for three weeks to establish the hyperlipidemic model. After 3 weeks, the hyperlipidemic rats were took the blood sample from tail vein to measure the lipids and subdivided into 4 groups (n = 6 in each group). (1) HFD group: HFD with 10 ml/kg/day distilled water; (2) FN-MC (2 g/kg) group: HFD with 10 ml/kg/day 0.2 g·ml⁻¹ FN-MC; (3) FN-MC (4 g/kg) group: HFD with 10 ml/kg/day 0.4 g·ml⁻¹ FN-MC; (4) PDC group: HFD treated with 10 ml/kg/day 0.4 g·ml⁻¹ simvastatin solution. Rats of NFD group were given the same volume of distilled water equivalent to body weight. Distilled water and drugs were given by gavage once a day for 3 weeks.

**Sample collection**

During the experimental period, body weight was recorded once a week and the food intake of each group was recorded daily. After 3 weeks, blood from each rat was collected from caudal vein after an over-night fasting. The collected whole blood was kept in refrigerator in 4 °C for 15 min, then serum was obtained after the blood was centrifuged (3000 rpm for 15 min) and stored at −80 °C until analysis. The rats were sacrificed by cervical dislocation and tissues were harvested and stored at −80 °C until use.

**Serum and hepatic biochemical parameters analysis**

The serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufactures’ instructions. The serum apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) were measured by commercial kits (Westang Bio-Tech, Shanghai, China) according to the manufacturer’s protocols. A part of liver was homogenized in ice cold PBS. The homogenate was centrifuged and the supernatant was taken to measure the activity of SOD (xanthine oxidase method) and the content of MDA (TBA method, thiobarbituric acid method), by using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufactures’ instructions.
Histological analysis
Liver tissues were removed quickly from rats, fixed in 4% paraformaldehyde solution, and then embedded in paraffin. Sections were obtained and later stained with hematoxylin and eosin (H&E) for the histological examination under microscopic

Ultrastructural analysis
Liver tissues were isolated from rats, fixed with 2.5% glutaraldehyde solution and 1% osmium acid solution, then dehydrate with a different concentration gradient of ethanol solution and embedded with epoxy resin. The samples were treated with ultrathin microtome and then stained with uranyl citrate-lead citrate. Finally, the ultrastructure of hepatocytes was observed and photographed with transmission electron microscope. Total 36 planes (6 planes per group) were used to determine the surface area of lipid droplets. The surface area of lipid droplets was measured by using Image J software (National Institute of Mental Health, USA).

Statistical analysis
Values are presented as the mean ± S.E.M. Significant differences among the results of five groups were analyzed by GraphPad Prism 7.0 software using one-way ANOVA followed by Dunnett’s posttest. P < 0.05 was considered to be statistically significant.

Results
Effect of FN-MC on body weight, weight gain and food intake of rats
The body weight and the weight gain of rats are shown in Table 1 and Fig. 1. There were no differences in initial body weight among 5 groups in the experiment (P > 0.05), indicating that the grouping was reasonable. During the gavage period, the body weight of the rats increased with time, and the weight gain of the rats in HFD group was significantly higher than that of the other four groups. The increment of the rats in FN-MC group (4 g/Kg) was much smaller than that in HFD group and FN-MC (2 g/Kg) also had some effect on body weight. After 1 week of gavage, the body weights of the PDC group and FN-MC group were significantly different from that of HFD group (P < 0.05). Gavage for the second and the third week, compared with HFD group, the body weight of FN-MC (4 g/Kg) group had a significant difference (P < 0.01). After 4 weeks, although rats treated with FN-MC (2 g/kg) and FN-MC (4 g/Kg) were fed the high-fat diet, they had a reduced weight gain compared with HFD group (P < 0.05, P < 0.001, respectively). Meanwhile, the weight gain of PDC group after 4 weeks was also decreased compared with HFD group (P < 0.05). After 6 weeks, the total weight gain of FN-MC (4 g/Kg) group was much less than PDC group. However, food intake was no difference between the groups (Table 1). The results suggested that FN-MC reduced the weight gain of mice and had no significant effects on food intake.

Effect of FN-MC on serum lipid profiles of rats
Effects of FN-MC on serum lipid profiles in the experimental rats are shown in Fig. 2. After 3 weeks, the levels of TC and TG in the HFD group were significantly higher than those in NFD group (P < 0.01). But after treatment with 2 g/Kg and 4 g/Kg of FN-MC for 3 weeks, compared with HFD group, the levels of TC were significantly decreased by 14.86% (P < 0.05) and 28.87% (P < 0.01), and the levels of TG were significantly decreased by 22.78% (P < 0.05) and 34.86% (P < 0.01). The above results indicated that FN-MC had obvious hypolipidemic effect on hyperlipidemia rats and the 4 g/Kg of FN-MC had more capacity to lower the serum lipid profiles.

Effect of FN-MC on serum lipoprotein profiles of rats
Effects of FN-MC on serum lipoprotein profiles in the experimental rats are shown in Fig. 3. After 3 weeks, the levels of LDL-C in the HFD group were significantly higher than those in NFD group (P < 0.01), and the level of HDL-C was significantly decreased (P < 0.01). After treatment with FN-MC (2 g/Kg) for 3 weeks, compared with HFD group, the levels of LDL-C were significantly decreased by 18.22% (P < 0.05) and the level of HDL-C was significantly increased by 52.80% (P < 0.05). But after treatment with FN-MC (4 g/Kg) for 3 weeks, the levels of LDL-C were significantly decreased by 32.70% (P < 0.01) and the level of HDL-C was significantly increased.

| Table 1 | Effect of FN-MC on body mass and food intake of rats (n = 6) |
|--------|-------------------------------------------------------------|
| Group  | Original | Week-3 | Week-4 | Week-5 | Week-6 | Food intake (g/d) |
| NFD    | 317.48 ± 20.25 | 427.24 ± 40.36 | 449.92 ± 13.15* | 454.45 ± 11.81** | 457.80 ± 13.99** | 23.43 ± 3.62 |
| HFD    | 313.67 ± 16.15 | 433.44 ± 26.80 | 508.62 ± 35.15 | 523.35 ± 31.78 | 530.25 ± 32.00 | 21.22 ± 3.39 |
| FN-MC (2 g/kg) | 312.43 ± 14.69 | 432.57 ± 34.47 | 466.3 ± 19.13* | 478.51 ± 19.64* | 484.39 ± 23.57* | 21.85 ± 4.15 |
| FN-MC (4 g/kg) | 309.28 ± 19.77 | 436.96 ± 26.93 | 466.3 ± 19.13* | 478.51 ± 19.64* | 484.39 ± 23.57* | 21.85 ± 4.15 |
| PDC    | 304.41 ± 24.96 | 433.7 ± 32.71 | 452.8 ± 14.37** | 466.97 ± 16.15* | 475.00 ± 17.47* | 23.51 ± 4.96 |

The results were represented as mean ± SEM in each group (n = 6 rats/group). *P < 0.05, **P < 0.01 significant differences compared to HFD group; *P < 0.05, **P < 0.01 significant differences compared to NFD group.
Fig. 1 Effect of FN-MC on body weight and weight gain of rats. In the induction phase, rats were separated into the NFD group (n = 6) and HFD group (n = 24). After 3 weeks, the 24 rats were randomly assigned to four groups (six rats/group) for solvent, FN-MC (2 g/Kg), FN-MC (4 g/Kg) or simvastatin solution (10 mg/kg/day) administration. *P < 0.05, **P < 0.01, ***P < 0.001 significant differences compared to HFD group; #P < 0.05, ##P < 0.01, ###P < 0.001 significant differences compared to NFD group.

Fig. 2 Effect of three-week FN-MC treatment on serum lipid profiles (TC and TG) of rats. *P < 0.05, **P < 0.01 significant differences compared to HFD group; #P < 0.05, ##P < 0.01 significant differences compared to NFD group. TC: total cholesterol; TG: triglyceride.
by 91.74% \((P < 0.01)\), indicating that 4 g/Kg of FN-MC had more obvious hypolipidemic effect on hyperlipidemia rats.

**Effect of FN-MC on serum ApoA1 and ApoB levels of rats**

As shown in Table 2, compared with NFD group, the level of ApoB in HFD group was significantly higher \((P < 0.01)\) and the level of ApoA1 in HFD group was significantly lower \((P < 0.05)\). Meanwhile, the ratio of ApoA1/ApoB in HFD group was significantly lower than NFD group \((P < 0.001)\). Compared with HFD group, FN-MC (2 g/Kg) group and FN-MC (4 g/Kg) group showed the decreased ApoB levels and increased ApoA1 levels \((P < 0.05)\). The ratios of ApoA1/ApoB in FN-MC (2 g/Kg) group and FN-MC (4 g/Kg) group were significantly higher \((P < 0.001)\). After treatment with PDC, the ratio of ApoA1/ApoB was increased compared with HFD group \((P < 0.001)\). The results indicated that FN-MC had regulatory effects on the apolipoprotein level of high-fat diet mice.

**Table 2** Effect of FN-MC on serum ApoA1 and ApoB levels of rats \((n = 6)\)

| Group        | ApoA1 (mmol/L) | ApoB (mmol/L) | ApoA1/ApoB |
|--------------|----------------|---------------|------------|
| NFD          | 0.55 ± 0.08*   | 0.34 ± 0.03** | 1.62 ± 0.04*** |
| HFD          | 0.31 ± 0.04*   | 0.61 ± 0.06** | 0.51 ± 0.06*** |
| FN-MC (2 g/Kg)| 0.43 ± 0.03*   | 0.46 ± 0.03*  | 0.93 ± 0.03*** |
| FN-MC (4 g/Kg)| 0.48 ± 0.03**  | 0.41 ± 0.05*  | 1.17 ± 0.04*** |
| PDC          | 0.49 ± 0.04**  | 0.41 ± 0.03*  | 1.20 ± 0.04*** |

The results were represented as mean ± SEM in each group \((n = 6\) rats/group). \(*P < 0.05, **P < 0.01, ***P < 0.001\) significant differences compared to HFD group; \(*P < 0.05, **P < 0.01, ***P < 0.001\) significant differences compared to NFD group.

**Effect of FN-MC on liver antioxidant capacity of rats**

As shown in Fig. 4, compared with NFD group, the content of MDA in the liver of HFD group was significantly increased \((P < 0.01)\), and the activity of antioxidant enzyme SOD was significantly decreased. Compared with HFD group, the content of MDA in the liver of FN-MC (2 g/Kg and 4 g/Kg) group was significantly decreased by 31.90\% \((P < 0.01)\) and 53.67\% \((P < 0.01)\) and the activity of SOD was significantly increased by 13.58\% \((P < 0.01)\) and 33.36\% \((P < 0.01)\), which was similar to that of PDC group. The results indicated that FN-MC had a significant improvement on the antioxidant ability of liver tissue of hyperlipidemic rats.

**Effects of FN-MC on morphology and ultrastructure of liver tissue in rats**

Effects of FN-MC on histopathological sections of liver tissue, ultrastructure of liver tissue and quantitative analysis of lipid droplets surface area are shown in Fig. 5 and 6. In the liver section of the NFD group, the structure of hepatic lobular was clear and intact. Hepatocytes were arranged in the hepatic cords (Fig. 5a). There were few lipid droplets in the hepatocytes, and a large number of mitochondria and endoplasmic reticulum (ER) were found in the cytoplasm. The mitochondria did not show swelling, and the RER was clear and dense. However, the liver tissues of HFD group were disordered and the liver cells have necrosis partly (Fig. 5b). There were a large number of lipid droplets that were disorderly arranged in liver cells (Fig. 5c). After the treatment with FN-MC, the liver tissues returned to normal status (Fig. 5d). In the cytoplasm, the lipid droplets reduced significantly and the mitochondria was rich...
but still swelling. At the same time, the number of RER and glycogen granules in the cytoplasm increased clearly which was similar with the PDC group (Fig. 6c, d, e and f). The results suggested that FN-MC reduced the fatty liver and lipid droplets, repaired liver damage caused by high-fat diet, and increased the number of mitochondria and ER in the cytoplasm.

**Discussion**

According to the existing study, long-term high-fat diet can lead to the increased blood lipid levels [17–19]. In our experiment, we establish hyperlipidemic rat models by feeding high fat diet to explore the therapeutic effect of FN-MC on hyperlipidemia. Our pharmacological test indicated that FN-MC was sufficient to reduce the TC, TG, LDL-c and ApoB levels and increase the HDL-c, ApoA1 and ApoA1/ApoB level in hyperlipidemic rats. Compared with HFD group, the content of MDA in the liver of FN-MC group was decreased and the activity of SOD of FN-MC group was increased. By observing the ultrastructure of liver tissue, we found that after the treatment with FN-MC, the lipid droplets were significantly reduced and the number of ER in the cytoplasm increased. These results suggested that FN-MC exerted hypolipidemic and antioxidant effects and may have a protective effect against atherosclerosis which is similar to simvastatin, the positive drug [20–22]. In addition, after 6 weeks of gavage, the weight gain of the FN-MC group was significantly lower than that of the HFD group, indicating that FN-MC has anti-obesity effects.
It is well established that long-term high blood lipids can lead to liver fibrosis; high-fat diet can induce fatty liver and liver damage. Mitochondria are organelles that provide ATP for life activities. Cell energy metabolism also relies heavily on its beta oxidation of fatty acids. It has been reported that hyperlipidemic patients have ultrastructural mitochondrial changes. Mitochondrial dysfunction can impair the homeostasis of fatty liver and induce excessive production of ROS, thereby triggering lipid peroxidation, cytokine release and cell death. [23] ER is responsible for lipid, protein synthesis, modification and transport. Studies have shown that the structural and functional abnormalities of ER are associated with hyperlipidemia. Through the observation and comparison of liver tissue sections, we confirmed that rats of HFD group had increased lipid droplets, large amounts of liver damage, reduced mitochondria and endoplasmic reticulum. After treatment with FN-MC, the fatty liver was reduced, lipid droplets were significantly reduced, liver damage caused by high-fat diet was repaired, and the number of mitochondria and ER in the cytoplasm was increased.

Changes in blood lipid levels are major risk factors for lipid metabolism disorders; elevated serum TC, TG and LDL-C levels and decreased HDL-C levels are major symptoms of hyperlipidemia [24]. Consistent with previous studies, the high-fat diet induced elevated levels of serum TC, TG, and LDL-C in the HFD group [25]. Serum LDL-C and TG levels are risk indicators for atherosclerotic cardiovascular disease. [26] Lowering serum LDL-C and TG levels may improve the risk of vascular disease and reduce the incidence of acute coronary events. [27] In our study, serum TC, TG, LDL-C levels were significantly lower in the FN-MC group compared with the HFD group, while HDL-c levels were significantly elevated, similar to the PDC group.

Apolipoprotein is a protein component that constitutes plasma lipoprotein which is to transport lipids and stabilize lipoproteins. ApoA1 is mainly derived from HDL-C, and ApoB is mainly derived from LDL-C. Therefore, decreased ApoA1 and increased ApoB are the manifestation of hyperlipidemia. ApoA1/ApoB is a risk factor for atherosclerotic cardiovascular disease [28]. The results of this study showed that FN-MC increased
**Conclusion**

The results suggested that FN-MC could prevent weight gain, reduce serum levels of lipid profiles, prevent hepatic fatty deposition effectively. Meanwhile, FN-MC increased the activity of antioxidant enzymes, decreased lipid peroxidation and exerted antioxidant properties, possibly preventing the progress of hyperlipidemia. Therefore, FN-MC is a potential food additive or pharmaceutical agent to treat or prevent hyperlipidemia. Further studies of hyperlipidemia, antioxidant and anti-obesity mechanism of FN-MC are necessary.

**Abbreviations**

ApoA1: Apolipoprotein A1; ApoB: Apolipoprotein B; FN-MC: Fragaria nilgerrensis Schlecht.; HDL-C: High-density lipoprotein; HFD: High-fat diet; LDL-C: Low-density lipoprotein; MDA: Hepatic malondialdehyde; NFD: Normal-fat diet; PDC: Positive drug control; simvastatin; SOD: Hepatic Superoxide Dismutase; TC: Total cholesterol; TG: Triglyceride.

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**Availability of data and materials**

All data generated or analyzed during this study are included within the article.

**Authors’ contributions**

L.G., Z.L., Y.L. and L.W. designed the study; Z.L., Y.L. and L.Z. analyzed the biochemical data; X.W., X.L. and L.Z. analyzed the Histopathological data. Y.L. and L.Z. drafted the manuscript. All authors read and approved the final manuscript.

**Ethics approval**

All the studies were approved by the Institutional Animal Care and Use Committee of East China Normal University.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interest.

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