**Regular Article**

The Anti-hyperlipidemia Effects of Raw *Polygonum multiflorum* Extract in Vivo

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**Polygonum multiflorum** is widely used in the prevention and treatment of hyperlipidemia in traditional Chinese Medicine. In this study, the effects and relevant mechanisms of lipid-regulation by raw *Polygonum multiflorum* (RPM) were investigated. The results indicated that the basal plasma lipids, such as low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG), were significantly decreased in RPM treatment groups compared with the model group, especially in the RPM high dose group. The key enzymes involved in lipid metabolism, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) in plasma were generally reduced after oral administration, which was consistent with the transcription levels of their target genes. In addition, the hepatotoxicity of RPM was investigated, and RPM showed slightly less liver injury than that induced by simvastatin. Historical analysis indicated that the fat vacuoles and steatosis in hepatocytes were relieved after oral administration of RPM extract at a high dose of 16.2 g/kg, which was more obvious than that induced by simvastatin. These results revealed that RPM exerted its lipid-lowering effect by regulating the expression of related genes, and performed better than simvastatin in the treatment of hyperlipidemia.

**Key words** raw *Polygonum multiflorum*; hyperlipidemia; lipid-lowering; hepatotoxicity

Hyperlipidemia is a common metabolic syndrome characterized by increased total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) levels, which facilitate endothelial dysfunction and atherogenesis, the major risk factor for cardiovascular disease. Statins are commonly used in clinical treatment as first-line lipid-lowering drugs, especially in the treatment of hyperlipidemia. They act to suppress the activities of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and thus affect the formation of mevalonic acid, the rate-limiting step in the biosynthesis of cholesterol, but this treatment is often accompanied by adverse reactions such as gastrointestinal tract issues, myopathy and hepatotoxicity. Traditional Chinese Medicine (TCM) is a healthcare-focused medical system based on more than 3000 years of continuous practice experience, and therefore has its own advantages and characteristics in early intervention and personalized treatment. Some Chinese medicinal herbs have shown lipid-lowering effects by reducing the absorption of exogenous lipids and the synthesis of endogenous lipids, and also by facilitating the transportation, metabolism and excretion of lipids.

TCM performs multi-target network regulation based on the function of the whole body; between its characteristic good efficiency and low toxicity, TCM has received extensive attention all over the world. There are various Chinese medicinal herbs clinically prescribed to cure hyperlipidemia. Among these herbs, *Polygonum multiflorum* ranked as the fifth most frequently used crude drug. Recent studies have demonstrated that the extract of *Polygonum multiflorum THUNB.* (PMP, which was originated from RPM steamed black beans) feature a history of treatment in the cure of hyperlipidemia. *RPM* exerted the most beneficial effects. Since processing decreases the contents of some ingredients correlated with its hypolipidemic effect, for instance, its toxicity and efficacy were studied separately, and again lack systematic research. Therefore, it is necessary to now investigate both the lipid-lowering effect and hepatotoxicity of RPM extract simultaneously.

In this study, we have evaluated the lipid-lowering effect and revealed the relevant mechanism of RPM extract in rats fed a high fat diet (HFD). At the same time, its hepatotoxicity was investigated during our experiments.

**MATERIALS AND METHODS**

**Chemicals** Simvastatin (Hangzhou MSD Pharmaceutical Co., Ltd., China) was used as a positive control. Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Nanjing Jiancheng Bioengineering Institute. SYBR Green PCR kit, RNAPrep Pure Tissue kit and FastQuant reverse transcription kit came from Tiangen Biotech Co., Ltd.

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(Beijing). The primer was provided by Sangon Biotech Co., Ltd. (Shanghai).

**Extraction of RPM** RPM was purchased from Beijing San He Co., Ltd. (Beijing, China). Air-dried, powdered RPM (43 kg) was extracted with 70% ethanol (430 L×1.5 h×3). The extraction was combined, condensed and lyophilized to yield 5.4 kg RPM powder. The final RPM powder was used as a therapeutic drug for hyperlipidemia rats.

**Chromatographic Analysis of RPM** RPM extract was analyzed by HPLC using an Agilent 1260 Infinity (America) gradient liquid chromatograph. A poroshell 120 EC-C18 column (50 mm×4.6 mm×5 μm) was used. Column temperature was kept at 35°C, and a 5 μL sample was injected into the column and eluted with a constant flow rate of 1.0 mL/min. The UV detection wavelength was set at 254 nm and the main components of RPM extract were determined. The contents of 2,3,5,4'-tetrahydroxy-stilbene-2-O-β-D-glucoside, emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, emodin, chrysophanol and physcion were 2.93, 0.16, 0.01, 0.06, 0.006 and 0.04%, respectively, as determined by HPLC analysis. The chromatograms of standards and RPM are listed in Supplementary Figure S1.

**Animals and Experimental Design** Thirty six male Sprague-Dawley (SD) rats were purchased from Beijing HFK Bioscience Co., Ltd., China. After one week of acclimation, rats (weighing 230–240 g) were randomly divided into 6 groups (Table S1) and fed a standard diet (C), a high-fat diet (M), a simvastatin-supplemented high-fat diet (S, 1.2 mg/kg), or a RPM extract-supplemented high-fat diet (M+PML, 2.7 g/kg; M+PMM, 8.1 g/kg; M+PMH, 16.2 g/kg) for 4 months. Groups C, M and S served as the blank control, model control and positive control group, respectively. Group M+PML, 2.7 g/kg; M+PMM, 8.1 g/kg; M+PMH, 16.2 g/kg was extracted with 70% ethanol (430 L) into 6 groups (Table S1) and fed a standard diet (C), a high-fat diet (M), a simvastatin-supplemented high-fat diet (S, 1.2 mg/kg), or a RPM extract-supplemented high-fat diet (M+PML, 2.7 g/kg; M+PMM, 8.1 g/kg; M+PMH, 16.2 g/kg) for 4 months. Groups C, M and S served as the blank control, model control and positive control group, respectively. Group M+PML, M+PMM and M+PMH served as RPM treatment groups. Standard diets and HFD20) which contained 2% cholesterol, 10% lard, 10% powdered egg yolk, 0.2% bile salt, and 78.8% standard diet were provided by Beijing HFK Bioscience Co., Ltd., China. The animals were maintained at a steady temperature and humidity (20–23°C, 50–60%) under a 12 h light/dark cycle. All animal procedures were conducted in accordance with the guidelines for Animal Experimentation of the China Academy of Chinese Medical Sciences.

Body weights were measured once a week to adjust the dosage. Rats were sacrificed at the end of the experiment. Liver tissue was rapidly excised, weighed, and stored at −80°C until use. Samples of the resected liver were later used for analysis of the histology, and in RT-PCR.

**Biochemical Parameters** Blood samples were collected from the retro-orbital venous plexus, in the amount of about 1.5 mL once a month. Plasma was then obtained by centrifugation (3500 rpm, 15 min, 4°C). Levels of LDL-C, HDL-C, TC, TG, alkaline phosphatase (ALP), total bile acid (TBA), alanine aminotransferase (ALT) and glutamate transaminase (AST) were quantitatively analyzed by an automatic biochemical analyzer (Toshiba 40- FR, Japan).

**Lipid Regulation Mechanisms Investigation** Plasma 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC) were tested by ELISA assay kits with a microplate reader (Tecan-Infinite 200 Pro NanoQuant, Switzerland). The protein expression of phosphorylated acetyl CoA carboxylase (p-ACC) and ACC in the liver of the rats were also tested by ELISA assay kits. Gene expression levels in liver tissues obtained from different groups were detected by quantitative real-time PCR. Then, 1 μg of total RNA was extracted for reverse transcription. The primer sequences of the target genes are listed in Table S2. In addition, 20 μL reaction volumes were used for data analysis using Rotor-gene Q Software (Qiagen). PCR reactions for each gene were repeated 3 times. The relative levels of each gene expression were determined by the 2−ΔACt method.

**Detection of Inflammatory Factor in Plasma** Tumor necrosis factor alpha (TNF-α) and glutathione-S-transferase alpha (GST-α) were determined in plasma using commercial ELISA assay kits with a microplate reader (Tecan-Infinite 200 Pro NanoQuant, Switzerland).

**Histological Analysis** Each liver was fixed in 10% neutral-buffered formalin and processed routinely for embedding in paraffin. Tissue sections (3 μm) were stained with hematoxylin and eosin (HE) and examined under a light microscope.

**Statistical Analysis** All data in this study are expressed in the form of mean±standard deviation (S.D.) The data were evaluated by one-way ANOVA, and the differences between means assessed using Duncan’s test with a significance level of p<0.05 and p<0.01.

**RESULTS**

**Effects of RPM Extract on the Physical Condition of Rats** Body weight can directly reflect a lipid-lowering effect. We found that the body weights in the model group were generally higher than in the control group, especially in the last month (Table 1); however, they were remarkably decreased in RPM treatment groups. The high dose of RPM extract exhibited a significant weight loss effect, and the weight was returned to normal over time; moreover, liver weight gave similarly consistent results. Simvastatin did not have the ability to reduce body and liver weight. There was no significant difference in food intake or feces between the RPM treatment and control groups. Plasma was then obtained by centrifugation (3500 rpm, 15 min, 4°C). Levels of LDL-C, HDL-C, TC, TG, alkaline phosphatase (ALP), total bile acid (TBA), alanine aminotransferase (ALT) and glutamate transaminase (AST) were quantitatively analyzed by an automatic biochemical analyzer (Toshiba 40- FR, Japan).

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<table>
<thead>
<tr>
<th>Groups</th>
<th>0 Month (g)</th>
<th>1 Month (g)</th>
<th>2 Months (g)</th>
<th>3 Months (g)</th>
<th>4 Months (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>239.00±8.87</td>
<td>378.33±9.95</td>
<td>431.67±21.75</td>
<td>490.77±22.67</td>
<td>526.00±29.02</td>
</tr>
<tr>
<td>M</td>
<td>248.95±3.51</td>
<td>431.50±24.32**</td>
<td>494.73±20.77**</td>
<td>539.48±25.70*</td>
<td>603.00±31.26**</td>
</tr>
<tr>
<td>S</td>
<td>245.78±5.50</td>
<td>422.65±17.81**</td>
<td>490.75±25.06**</td>
<td>572.67±35.43**</td>
<td>620.18±45.04**</td>
</tr>
<tr>
<td>M+PML</td>
<td>243.62±5.44</td>
<td>410.17±14.71</td>
<td>477.37±8.05</td>
<td>546.55±15.86</td>
<td>582.96±15.01</td>
</tr>
<tr>
<td>M+PMM</td>
<td>244.77±5.92</td>
<td>410.12±17.78</td>
<td>480.16±28.58</td>
<td>542.12±42.11</td>
<td>589.84±43.56</td>
</tr>
<tr>
<td>M+PMH</td>
<td>245.14±9.14</td>
<td>375.71±9.49*</td>
<td>444.46±14.85*</td>
<td>497.94±18.46*</td>
<td>539.07±25.38**</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D. (n=6). *p<0.05, **p<0.01 compared with the control group. *p<0.05, **p<0.01 compared with the model group.
groups and the model group (Table S3).

Effects of RPM Extract on Lipid Regulation in Plasma
The biochemical index clearly reflected the effects of RPM extract on lipid regulation. Plasma levels of LDL-C, TC, TG were consistently increased in the model group compared with the blank control group. LDL-C levels in the model group were increased to the highest level in the last month, to 4 times that in blank control group, whereas it was reduced in RPM treatment groups (Table 2). In addition, we found that RPM extract exerted a stable and fast lipid-lowering effect in the initial treatment at a high dose. A similar regulating effect of RPM extract was found in TC-regulation (Table 3). The TG content was decreased in RPM treatment groups, but the regulation of TG (Table 4) was not as significant as LDL-C.

Table 2. Effects of RPM Extract on the Levels of LDL-C in Plasma of Rats

<table>
<thead>
<tr>
<th>LDL-C (mmol/L)</th>
<th>0 Month</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
<th>4 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.36±0.13</td>
<td>0.43±0.08</td>
<td>0.37±0.05</td>
<td>0.35±0.06</td>
<td>0.32±0.04</td>
</tr>
<tr>
<td>M</td>
<td>0.40±0.05</td>
<td>0.56±0.07*</td>
<td>0.61±0.09**</td>
<td>0.89±0.12**</td>
<td>1.20±0.15**</td>
</tr>
<tr>
<td>S</td>
<td>0.37±0.08</td>
<td>0.58±0.17</td>
<td>0.47±0.11*</td>
<td>0.65±0.12**</td>
<td>0.63±0.15**</td>
</tr>
<tr>
<td>M+PML</td>
<td>0.41±0.08</td>
<td>0.51±0.04</td>
<td>0.49±0.07*</td>
<td>0.71±0.10*</td>
<td>0.66±0.19**</td>
</tr>
<tr>
<td>M+PMM</td>
<td>0.39±0.04</td>
<td>0.62±0.09</td>
<td>0.46±0.04**</td>
<td>0.65±0.11*</td>
<td>0.70±0.14**</td>
</tr>
<tr>
<td>M+PMH</td>
<td>0.40±0.06</td>
<td>0.53±0.09</td>
<td>0.50±0.15</td>
<td>0.52±0.14**</td>
<td>0.50±0.17**</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D. (n=6). *p<0.05, **p<0.01 compared with the control group. 

Table 3. Effects of RPM Extract on the Levels of TC in Plasma of Rats

<table>
<thead>
<tr>
<th>TC (mmol/L)</th>
<th>0 Month</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
<th>4 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.33±0.06</td>
<td>1.40±0.21</td>
<td>1.37±0.13</td>
<td>1.22±0.09</td>
<td>1.34±0.14</td>
</tr>
<tr>
<td>M</td>
<td>1.39±0.51</td>
<td>1.65±0.20*</td>
<td>2.04±0.33**</td>
<td>2.41±0.46**</td>
<td>3.55±0.36**</td>
</tr>
<tr>
<td>S</td>
<td>1.43±0.43</td>
<td>1.49±0.25</td>
<td>1.57±0.17*</td>
<td>1.67±0.14**</td>
<td>1.84±0.38**</td>
</tr>
<tr>
<td>M+PML</td>
<td>1.39±0.07</td>
<td>1.63±0.11</td>
<td>1.61±0.07*</td>
<td>1.71±0.16*</td>
<td>1.87±0.41**</td>
</tr>
<tr>
<td>M+PMM</td>
<td>1.40±0.18</td>
<td>1.61±0.35</td>
<td>1.63±0.19*</td>
<td>1.66±0.04*</td>
<td>1.92±0.17**</td>
</tr>
<tr>
<td>M+PMH</td>
<td>1.38±0.10</td>
<td>1.56±0.12</td>
<td>1.50±0.18**</td>
<td>1.47±0.26**</td>
<td>1.69±0.30**</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D. (n=6). *p<0.05, **p<0.01 compared with the control group. 

Table 4. Effects of RPM Extract on the Levels of TG in Plasma of Rats

<table>
<thead>
<tr>
<th>TG (mmol/L)</th>
<th>0 Month</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
<th>4 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.67±0.06</td>
<td>0.69±0.04</td>
<td>0.70±0.22</td>
<td>0.82±0.15</td>
<td>0.96±0.19</td>
</tr>
<tr>
<td>M</td>
<td>0.69±0.09</td>
<td>0.81±0.09*</td>
<td>0.95±0.17*</td>
<td>1.26±0.33**</td>
<td>1.53±0.13**</td>
</tr>
<tr>
<td>S</td>
<td>0.61±0.09</td>
<td>0.70±0.19</td>
<td>0.53±0.10**</td>
<td>0.84±0.09**</td>
<td>1.26±0.33</td>
</tr>
<tr>
<td>M+PML</td>
<td>0.56±0.15</td>
<td>0.70±0.05*</td>
<td>0.70±0.13*</td>
<td>1.33±0.19</td>
<td>1.41±0.09</td>
</tr>
<tr>
<td>M+PMM</td>
<td>0.67±0.22</td>
<td>0.78±0.19</td>
<td>0.67±0.10**</td>
<td>1.20±0.19</td>
<td>1.30±0.09*</td>
</tr>
<tr>
<td>M+PMH</td>
<td>0.59±0.06</td>
<td>0.71±0.11</td>
<td>0.59±0.13**</td>
<td>0.98±0.20*</td>
<td>1.02±0.28**</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D. (n=6). *p<0.05, **p<0.01 compared with the control group. 

Fig. 1. Effects of RPM Extract on the Expression of the Key Enzymes Involved in Lipid Metabolism, Including HMGR (A), FAS (B) and ACC (C) in Plasma of Hyperlipidemic Rats

Values are given as mean±S.D. (n=6). **p<0.01 compared with the control group. *p<0.05, **p<0.01 compared with the model group.
and TC. The results also indicated that the high concentration of RPM extract showed an advantage in its LDL-C, TC and TG-lowering effect compared with that by simvastatin. However, the HDL-C level was not changed in the RPM treatment groups relative to the model group (Table S4).

**Lipid-Regulating Mechanisms of RPM Extract** To investigate the potential mechanism of RPM in the treatment of hyperlipidemia, the activities of HMGR, ACC and FAS in plasma, as well as the relative expression levels of its target gene in liver, were tested by ELISA kits and RT-PCR, respectively. The results indicated that HFD enhanced the levels of HMGR, ACC and FAS by 52, 57, and 60%, respectively (Fig. 1). Low, medium and high doses of RPM extract all showed down-regulation effects on HMGR, ACC and FAS. Additionally, the high concentration of RPM treatment group resulted in a significant down-regulation of the expression levels of HMGR, ACC, FAS and SREBP-1c mRNA in the liver by 37, 54, 62, and 70%, respectively, which was correlated with enzyme activity results; thus its regulating effect was better than simvastatin at a high dose (Fig. 2). Also, the ratio of p-ACC to ACC in liver was significantly reduced in the model group compared with the blank control group, whereas it was increased in RPM treatment groups and the positive control group. The higher the dose of the RPM extract, the greater the proportion of p-ACC to ACC (Fig. 3).

**Histological Analysis** Fatty degeneration, inflammation, necrosis and cytoplasmic vacuoles in hepatocytes were observed in the model group, while no histological abnormalities were found in the blank control group (Fig. 4). Simvastatin could control the progression of fatty degeneration, while it did not alleviate the inflammatory reaction. Additionally, a medium dose of RPM extract exhibited a therapeutic effect by reducing hepatic steatosis, whereas the high dose of RPM extract not only markedly reduced the hepatic steatosis but also alleviated inflammation. There was no significant difference between the model group and low dose RPM treatment group. Histopathological signs of inflammation and fatty

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**Fig. 2.** Effects of RPM Extract on Gene Expression Involved in Lipid Metabolism, Including HMGR mRNA (A), FAS mRNA (B), ACC mRNA (C) and SREBP-1c mRNA (D) in the Liver of Hyperlipidemic Rats

Values are given as mean±S.D. (n=6). **p<0.01 compared with the control group. *p<0.05, **p<0.01: compared with the model group.

**Fig. 3.** Effects of RPM Extract on the Protein Expression of ACC in the Liver of Hyperlipidemic Rats

The ratio of ACC and its phosphorylated form (p-ACC) were evaluated by ELISA kits. Values are given as mean±S.D. (n=6). *p<0.05, **p<0.01 compared with the model group.
degeneration in the liver were scored semi-quantitatively and referred to Brunt’s criteria22) and other references.23) Three typical fields of vision were randomly chosen under \( \times 100 \) magnification. Steatosis was graded 0–3 (0, the percentage of steatosis is less than 5%; 1, the percentage of steatosis cells is between 5 and 33%; 2, the percentage of steatosis is between 34 and 66%; 3, the percentage of steatosis is more than 66%), as shown in Table 5. The portal area inflammation was graded 0–3 (0 is none, 1 is mild; 2 is moderate; 3 is severe), also as shown in Table 5. The cellular components of inflammation (lymphocytes) were noted (Figure S2).

**Study on Liver Toxicity** The results above indicate that RPM extract has a remarkable lipid-lowering effect, especially at the high dose. However, the adverse hepatic reaction of RPM and its preparations has increasingly been reported.18,19) Thus, we investigated the hepatotoxicity of RPM extract comprehensively in this study.

**Effects of RPM Extract on Plasma Biochemical Indexes** ALP, TBA, ALT and AST were commonly used to evaluate liver function. The contents of ALP and TBA increased by 3- and 2.6-fold, respectively, in the model group in the last month (Table S5, Table S6), which was markedly higher compared with the blank control group, whereas levels in the RPM treatment groups were markedly decreased. Simvastatin

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**Table 5. The Effects of RPM Extract on Histopathological Changes**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>M</th>
<th>S</th>
<th>M+PML</th>
<th>M+PMM</th>
<th>M+PMH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty generation (grade)</td>
<td>0</td>
<td>2.83±0.41**</td>
<td>2.33±0.52*</td>
<td>2.00±0.71*</td>
<td>1.80±0.84*</td>
<td>1.00±0.00##</td>
</tr>
<tr>
<td>Inflammation (grade)</td>
<td>0</td>
<td>3.00±0.00**</td>
<td>2.00±1.10*</td>
<td>2.20±1.10*</td>
<td>2.60±0.89</td>
<td>1.33±0.82##</td>
</tr>
</tbody>
</table>

**p<0.01 compared with the control group. *p<0.05, **p<0.01 compared with the model group.**
could reduce the ALP and TBA levels, but the contents of ALP remained at a higher level compared with that of the high concentration of RPM treatment group, which implied that simvastatin should not be taken for a long time. At the same time, ALT and AST showed no significant change (data not shown).

Effects of RPM Extract on the Inflammatory Factor and Detoxifying Enzyme in Plasma To corroborate whether the liver was damaged, levels of TNF-α and GST-α were evaluated. The levels of TNF-α and GST-α increased 54% and 91%, respectively, in the model group compared with the blank control group (p<0.01, Fig. 5). RPM treatment resulted in a remarkable reduction in TNF-α and GST-α content, especially at the high concentration, which was similar to the results in the regulation of ALP and TBA. Simvastatin could reduce the levels of TNF-α and GST-α, but these remained at a higher level compared with the high concentration of RPM treatment group, which implied that simvastatin was not suitable for long-term use.

DISCUSSION

Hyperlipidemia is a common metabolic syndrome characterized by increased TC, TG, and LDL-C, and decreased HDL-C levels. In animals, it facilitates the occurrence of lipid metabolism disorders. High lipid levels present a risk factor for hypertension, coronary heart disease and cerebrovascular damage. Polygonum multiflorum is widely used in Asia for its venous and arterial effects. Although the lipoprotein-lowering effect of Polygonum multiflorum has been previously confirmed, there were few systematic studies of the mechanism of Polygonum multiflorum. In this study, we evaluated the effects of RPM extract on a high-fat diet-induced hyperlipidemia model in rats.

In order to explore further the effect of RPM extract on high-fat diet-induced hyperlipidemia, rats were fed a high-fat diet (HFD) after being divided into four groups: control, model, RPM treatment, and simvastatin treatment groups. The model group rats were fed a HFD, but decreased after treatment with RPM. Thus, we investigated the expression of HMGR, ACC, FAS, and SREBP-1c mRNA in the model group compared with the control group. We found that they were all down-regulated in RPM treatment groups. Therefore, the mechanism of RPM extract’s lipid lowering effect in vivo might be the suppression of the expression of HMGR and ACC, thus affecting the biosynthesis of TC and TG.

Aside from the disorder of lipid metabolism in hyperlipidemia rats, an inflammatory reaction in the liver was observed. Simvastatin was able to decrease the contents of ALP, TBA, TNF-α and GST-α, but the high concentration of RPM extract exhibited even greater effects. Thus, the hepatotoxicity caused by RPM extract was less than that caused by simvastatin. Histopathological findings in the liver showed that there was inflammatory infiltration around portal areas (Figure S2). Lymphocytes were noted. The inflammation score in rats fed a high-fat diet alone was significantly higher than in rats in the control group. However, the inflammation score was significantly lower after treatment with RPM. Thus, there were no significant changes in ALT and AST plasma levels in rats during the experiment, although liver injury did occur according to histological analysis. Thus, we need to find new indicators. GST-α was mainly found in centrolobular cells in a high concentration, thus this metabolic zone of the liver was more sensitive to injury. Therefore, TNF-α is a typical feature in animal models of hepatic injury and inflammation. The proinflammatory cytokine TNF-α is the central mediator of apoptosis and necrotic liver damage. Therefore, we can obtain more useful information by examining changes in TNF-α and GST-α. Long-term feeding of a high-fat diet to rats may increase TNF-α and GST-α levels, resulting in lipid metabolism disorder and inducing inflammation. However, RPM was shown to reduce TNF-α and GST-α levels and demonstrated an anti-inflammatory effect that helped to regulate lipid levels.

In summary, the results of this study indicate that RPM extract may play a significant role in the treatment of hyperlipidemia by inhibiting the expression of HMGR, ACC and FAS,
and by down-regulating the transcription levels of its target genes. In addition, RPM extract could alleviate the inflammation caused by a high-fat diet.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**REFERENCES**


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