Comparison of anti–atherosclerotic effects of two different extracts from leaves of *Mallotus furetianus*

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**Abstract**

**Objective:** To compare the anti–atherosclerotic effects of two different extracts from the leaves of *Mallotus furetianus* by using rat model of atherosclerosis. **Methods:** The air-dried powdered *Mallotus furetianus* leaves were extracted with ethanol and then evaporated. The ethanol extract was experienced Diaion HP-20 CC with a gradient of MeOH and H₂O (50 division 50, 100 division 0, v/v) and two fractions, *Mallotus furetianus* A (Mf A) and *Mallotus furetianus* B (Mf B) were obtained. Rats were divided into control, atherosclerosis and vitamin E, Mf A and Mf B treated groups. Atherosclerotic model was established by administrating a loading dose of vitamin D₃ and feeding standard diet enriched with 2% cholesterol, 0.5% porcine cholate, 0.2% methimazole, 5% sugar, 10% pork fat. Vitamin E (0.20 g/kg), Mf A (0.053 g/kg), Mf B (0.057 g/kg) (with the potential) were administered to interfere with the development of atherosclerosis. After 9 weeks, rats were sacrificed and the blood lipid as well as composition of bile was examined. In addition, the thoracic aorta was harvested to evaluate histological changes and the intima–media thickness ratio. **Results:** Atherosclerosis model was successfully established, administration of vitamin E, Mf A and Mf B increased excretion of total bilirubin in bile, decreased triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) level, enhanced ratio of high density lipoprotein–cholesterol and LDL-C in blood, improved histological changes and diminished intima–media thickness ratio of thoracic aorta in atherosclerotic rats. As for the difference in anti–atherosclerotic effects between Mf A and Mf B, Mf A may be more powerful in declining TG level and Mf B may be more effective in decreasing TC level. **Conclusions:** The two different extracts, Mf A and Mf B can prevent the development of atherosclerosis, In detail, Mf A is more effective in regulating TG level and Mf B is more powerful in modulating TC level in atherosclerotic rats.

1. **Introduction**

Atherosclerosis plays a key role in the development of vascular disease as well as the related morbidity and mortality occurred in both developing and developed countries¹. It is a chronic inflammatory disease characterized by accumulation of cholesterol deposits in macrophages in large- and medium–sized arteries which may induce a proliferation of certain cell types within the arterial wall and result in endothelial dysfunction and inflammation. Consequently, atherosclerotic plaque is formed and leads to thrombosis which obstructs blood flow of vital organs, for example, brain and heart. In the development of atherosclerosis, hypercholesteremia and lipid peroxidation are believed to be critically involved. Currently, principle of treatment for atherosclerosis includes lowering down blood lipid and clearing oxygen free radicals with the potential to prevent progression of atherosclerosis. *Mallotus furetianus*, a kind of tropical plant, belongs to the family of Euphorbiaceae², it is a type of indigenous herb to Hainan Island of China which is used as a folk medicine by natives to treat cholecystitis. Many studies showed that leaves extracts of *Mallotus furetianus* played an important role...
role in anti-oxidation, anti-atherosclerosis and acted as cholagogue[3-5].

The study was designed to ascertain the detailed anti-atherosclerotic effects of two different extracts from leaves of Mallotus furetianus.

2. Materials and methods

2.1. Plant collection and preparation of the extracts

The leaves of Mallotus furetianus used in this study were collected from Hainan Island of China. The air-dried powdered leaves were extracted three times with ethanol. An ethanol extract was obtained with evaporation of the solvent under reduced pressure. The extract was experienced Diaion HP–20 CC with a gradient of MeOH and H2O (50 : 50, 100 : 0, v/v) and two fractions, Mallotus furetianus A (Mf A) and Mallotus furetianus B (Mf B) were obtained.

2.2. Animal husbandry

All experiments were performed with the unanimous approval from five members of the animal ethics committee of Hainan Medical College (China). A total of 75 wistar rats weighing between 200–300 g were purchased from experimental animal center of Changchun. The rats were housed at a temperature of (25±2)°C, with the relative humidity of (55±5)%, a 12 h light/dark cycle (6:00 am – 6:00 pm light), and food and water ad libitum. Rats were allowed to adapt to their environment for at least one week before the start of experiment.

2.3. Animal experiment

A total of 75 wistar rats were randomly divided into five groups with 15 rats in each of the following group, control, atherosclerosis, and vitamin E, Mf A, and Mf B treated groups. Rats in atherosclerosis, and vitamin E, Mf A, and Mf B treated groups were injected with a loading dose of vitamin D3 (0.20 g/kg), followed by being fed with standard diet enriched with 2% cholesterol, 0.5% porcine cholate, 0.2% methimazole, 5% sugar, 10% pork fat to establish atherosclerotic model[6]. Rats in control group were injected with sodium chloride and were fed with standard diet. Rats in vitamin E, Mf A, and Mf B treated groups were administrated vitamin E (0.20 g/kg), Mf A (0.053 g/kg), Mf B (0.057 g/kg), respectively to prevent development of atherosclerosis. Rats in control and atherosclerosis groups were given solvent. After nine weeks, rats were sacrificed and blood was collected to evaluate triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), ratio of high density lipoprotein–cholesterol (HDL–C) and low density lipoprotein–cholesterol (LDL–C) and bile was harvested to analyze total bilirubin (TB) and cholesterol (CHO) inside. Isolated thoracic aorta was fixed with formalin, dyed with haematoxylin and eosin stain, therefore histological changes and intima–media thickness ratio were detected.

2.4. Statistical analysis

Data are expressed as mean±SD for the indicated number of independently performed experiments. Statistical significance between means was analyzed by one–way ANOVA or the Student’s t test utilizing the SPSS 13.0 version. P < 0.05 was taken as statistically significant.

3. Results

3.1. Effects of two different extracts from the leaves of Mallotus furetianus on the composition of bile

Concentration of bile TB in atherosclerosis group was significantly declined compared with that in control group (P<0.01), and bile TB in vitamin E, Mf A and Mf B treated groups was enhanced compared with that in atherosclerosis group (P<0.01). Excretion of CHO in atherosclerosis group was lower compared with that in control group (P<0.01) and that in vitamin E, Mf A and Mf B treated groups showed no significant change compared with that in atherosclerosis group (P>0.05) (Table 1).

3.2. Effects of two different extracts from the leaves of Mallotus furetianus on blood lipid

In atherosclerosis group, the levels of TC, TG, and ratio of HDL–C and LDL–C were raised compared with those in control group (P<0.01), while, those in vitamin E, Mf A and Mf B treated groups were declined compared with those in atherosclerosis group (P<0.05, P<0.01). LDL–C level in atherosclerosis group was higher compared with that in control group (P<0.01), and that in vitamin E, Mf A and Mf B treated groups was lower compared with that in atherosclerosis group (P<0.05). The decrease of TC level in Mf B treated group was greater in value than that in Mf A treated group, and the decrease of TG level in Mf A treated group was greater in value than that in Mf B treated group (Table 2).

3.3. Effects of two different extracts from the leaves of Mallotus furetianus on histological changes

In atherosclerosis group, histological examination results showed enhanced thickness of tunica intima, calcification and necrotic tissues in tunica media layers. The thickening tunica media was raised and thoracic aorta lumen became partly obstructed. Thickness of tunica media shrank. The histological changes were improved in vitamin E, Mf A and Mf B treated groups (Figure 1–5). Intima–media thickness ratios in vitamin E, Mf A and Mf B treated groups were decreased (P<0.01) (Table 3).
Figure 1. Atherosclerotic morphology changes under light telescope in rat thoracic aorta in control group. Original modification x100.

Figure 2. Atherosclerotic morphology changes under light telescope in rat thoracic aorta in atherosclerosis group. Original modification x100.

Figure 3. Atherosclerotic morphology changes under light telescope in rat thoracic aorta in vitamin E treated group. Original modification x100.

Figure 4. Atherosclerotic morphology changes under light telescope in rat thoracic aorta in _Mf A_ treated group. Original modification x100.

Table 1
Effects of two different extracts from the leaves of _Mallotus furetianus_ on bile composition (mean±SD, n=15).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>TB (umol/mL)</th>
<th>CHO (mmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>80.52±15.34</td>
<td>0.98±0.20</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>–</td>
<td>30.40±14.20</td>
<td>0.52±0.09</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.20</td>
<td>71.04±10.13</td>
<td>0.41±0.12</td>
</tr>
<tr>
<td><em>Mf A</em></td>
<td>0.053</td>
<td>74.33±30.36</td>
<td>0.40±0.10</td>
</tr>
<tr>
<td><em>Mf B</em></td>
<td>0.057</td>
<td>70.44±22.14</td>
<td>0.38±0.07</td>
</tr>
</tbody>
</table>

**P<0.01 as compared with that in atherosclerosis group, ΔΔP<0.01 as compared to control group.

Table 2
Effects of two different extracts from the leaves of _Mallotus furetianus_ on blood lipid (mean±SD, n=15).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>HDL-C/ LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>1.64±0.45**</td>
<td>1.41±0.22**</td>
<td>0.70±0.17</td>
<td>0.09±0.06</td>
<td>6.54±1.55</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>–</td>
<td>3.90±0.68ΔΔ</td>
<td>1.80±0.46ΔΔ</td>
<td>1.28±0.05ΔΔ</td>
<td>1.75±0.55ΔΔ</td>
<td>0.68±0.24ΔΔ</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.20</td>
<td>2.18±0.32**</td>
<td>1.32±0.19**</td>
<td>0.72±0.42</td>
<td>0.50±0.30</td>
<td>1.64±0.53**</td>
</tr>
<tr>
<td><em>Mf A</em></td>
<td>0.053</td>
<td>2.71±0.62**</td>
<td>0.90±0.40**</td>
<td>0.94±0.08</td>
<td>0.52±0.08</td>
<td>2.02±1.12**</td>
</tr>
<tr>
<td><em>Mf B</em></td>
<td>0.057</td>
<td>2.65±0.55**</td>
<td>1.32±0.36**</td>
<td>1.00±0.37</td>
<td>0.63±0.30</td>
<td>1.56±0.58**</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01 as compared to atherosclerosis group, ^P<0.05, ΔΔP<0.01 as compared to control group.
## 4. Discussion

Hyperlipidemia is a risk factor for the development of atherosclerosis, hypercholesterolemia and high level of LDL, especially oxidized-LDL, play an important role in the progression of atherosclerosis[7]. Antihyperlipidemic and antioxidative therapies become the main treatments of atherosclerosis. In the present study, rats were administered with a loading dose of vitamin D3 at the beginning of experiment and then were followed by breeding with standard diet enriched with cholesterol and fat. Similar studies showed that in the body of atherosclerotic models established by the above mentioned method, lipid oxidation and attenuation of anti-oxidation capacity are present; oxidized-LDL can infiltrate endothelial cells and form foam cells. Vitamin D3 can injure endothelial cells and facilitate infiltration of oxidized-LDL[4]. In addition, oxidized-HDL impairs "reverse cholesterol transport" and anti-atherogenic ability of HDL[8]. Therefore they are responsible for aggression and atherosclerotic plaque formation of atherosclerosis[9].

The dynamics of antioxidant action of vitamin E in LDL particle has been studied in detail, antioxidant actions of vitamin E were proved to suppress progression of atherosclerosis[10,11], which was also confirmed by our previous study[4]. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a family of phylogenetically conserved proteins that acts as a nuclear transcription factors. It is a crucial factor in the aggression and progression of atherosclerosis[12-15], elevated level of oxidized LDL was also proved to induce NF-κB in unstable angina patient in vivo[16-25]. As mentioned before, NF-κB can be activated by oxidized-LDL; some antioxidants such as resveratrol—a polyphenol found in red wine[26], have been proved to be NF-κB inhibitor. Vitamin E, a confirmed antioxidant that can interfere oxidant modification of LDL, might reveal its anti-atherosclerotic effect by inhibiting NF-κB activation.

Extracts from the leaves of Mallotus furetianus had been proved to act as anti-oxidant, anti-atherosclerotic and cholagogue both in animal experiments and in clinical observations. Moreover, extracts from the leaves of Mallotus furetianus were powerful DDPH scavenger[3-5]. Results of the present study showed Mf A and Mf B could accelerate excretion of TB, diminish hyperlipidemia, and improve histological changes of thoracic aorta as well as shrink intima-media thickness ratio in atherosclerosis. Those results coincide with our previous studies. There are two pathways for cholesterol excretion. The majority of cholesterol is excreted in the form of bile acid and the minority part of it is excreted in its original form. The extracts did no significant influence on bile cholesterol, while they could decline TC level of blood significantly. It revealed that the extracts might inhibit absorption of cholesterol or raise bile acid synthesis. It had been proved that extracts from the leaves of Mallotus furetianus took part in anti-oxidation both in vivo and in vitro[3,4]. Extracts induced decline in TC and TG level, reduction in cholesterol deposits in arteries and anti-oxidative effects might be related to their role in anti-atherosclerosis. Rich in polyphenol extracts from the leaves of Mallotus furetianus[27] might suppress NF-κB activation which may be responsible for the molecular mechanism of their above mentioned role.

The results also showed different properties of Mf A and Mf B which may hint that the composition of Mf A and Mf B might differ. It is known that Mf A consists of 3-hydroxy-4, 5R-dimethyl-2(5H)-furanone, gallic acid, (6S, 9R)-roseoside, aviculin, (+)-lyoniresinol 3-O-β-L-rhamnopyranoside, (Z)-3-hexenyl-β-D-glucopyranosid, whereas Mf B consists of 3, 4, 8, 9, 10-pentahydroxy-dibenzo[b, d]pyran-6-one, friedelolin,

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>Intima-media thickness ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>0.04±0.01**</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>–</td>
<td>0.78±0.16**</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.20</td>
<td>0.10±0.04**</td>
</tr>
<tr>
<td>Mf A</td>
<td>0.053</td>
<td>0.35±0.19**</td>
</tr>
<tr>
<td>Mf B</td>
<td>0.057</td>
<td>0.37±0.25**</td>
</tr>
</tbody>
</table>

** P<0.01 as compared with that in atherosclerosis group, *P<0.01 as compared to control group.
β-sitosterol and friedelin. The different chemical compositions and components of Mf A and Mf B might explain their difference in detailed anti-atherosclerotic roles of them, although further studies are still wanted to address this issue, and detailed information about chemicals and their relationship with NF-κ B activation become our on-going work.

Conflict of interest statement

We declare that we have no conflict of interest.

References


