Antihyperlipidemic effect of *Ficus dalhousiae* miq. stem bark on Triton WR-1339 and high fat diet-induced hyperlipidemic rats

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**A B S T R A C T**

*Ficus dalhousiae* stem bark is used to treat cancer and hyperlipidemia in folklore practices. *F. dalhousiae* stem bark methanolic extract (250 and 500 mg/kg b. wt.) was evaluated for antihyperlipidemic activity in Triton WR-1339 and high fat diet-induced hyperlipidemic rats. *F. dalhousiae* extract significantly (*P* < 0.005) alter the serum TC, TG, LDL-C and HDL-C levels to near normal in Triton WR-1339 and high fat diet-induced hyperlipidemic rats. The liver total cholesterol and triglycerides were also significantly reduced after treatment with 250 and 500 mg/kg of *F. dalhousiae*. The result of this study indicates that *F. dalhousiae* has a significant potential to use as a natural antihyperlipidemic agent.

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**1. Introduction**

Metabolic disorders that involve elevations in any lipoprotein species are termed as hyperlipidemias or hyperlipoproteinemias. The disease is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein level and decreased high density lipoprotein levels [1,2]. It occurs mainly due to impaired lipid and lipoprotein metabolism which adversely affects the pathways of cholesterol transport as well. The lipid metabolism is largely affected by host of risk factors which include lack of physical activities, sedentary life style, and diet enriched with cholesterol saturated fats, obesity, age and hormonal dysregulation [3,4]. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular diseases. Hypertriglyceridemia and hypercholesterolemia are closely related to the development of ischemic heart disease [5,6].

Statins, fibrates, niacin and resins are mainly used for the treatment of cardiovascular diseases but these are linked with a number of adverse effects. The following are the main ill-effects: flushing, dry skin, diarrhoea, nausea, muscle-related complaints, such as gastric irritation, abnormal liver function, rhabdomyolysis, myalgia, cramps, and muscle weakness (i.e., myopathy) [7,8]. In addition to this statins provoke liver dysfunction and renal failure [9,10]. Therefore, the development of promising cholesterol-lowering treatment alternatives to allopathic drugs is of utmost importance.

Evaluation of phytochemicals as new drug candidates for treating hyperlipidemia is a hopeful attempt. A lot of inhibitors of cholesterol synthesis e.g., flavonoids [11,12] and dietary fiber [13] are originated from natural sources and efficiently lower blood cholesterol level. *Ficus dalhousiae* miq. belonging to the family Moraceae. These are very rare species and found in the Nilgiri mountains at the altitudinal range of 605–1370 m. It is a tree of 10 m high, bark brown in colour, leaves are simple and ovate. The plant is seen in moist deciduous forests, endemic to Southern Western Ghats. Wherever it grows, its population size is very small and probably that is the reason for its inclusion under the very rare category in the threatened plant list [14]. Fruit is used as cardiotonic, leaves and bark are mainly used in liver and skin diseases. Leaf juice posses antisynergic activity. Roots are antispasmodic. Bark is used to treat cancer and hyperlipidemia in folklore practice [15]. From the literature survey it has seen that *F. dalhousiae* bark has not been evaluated for any pharmacological activity. In leaves, antihyperglycemic [16], gastroprotective [17], *in vitro* antioxidant [18] activities have been reported. This study was aimed to evaluate the antihyperlipidemic effects of *F. dalhousiae* bark in Triton WR-1339 and high fat diet-induced hyperlipidemic rats.
2. Materials and methods

2.1. Plant material

*F. dalhousiae* stem bark were collected from the surrounding regions of Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram, Kerala during the month of February–March, 2014. The plant was identified with the help of JNTBGRI taxonomist Dr. T. Sabu. A voucher specimen has been deposited at the herbarium of the institute.

2.2. Preparation of plant extracts

The collected barks of *F. dalhousiae* were chopped, dried and powdered. The powder (2 kg) was subjected to soxhletation process for 72 h at room temperature using methanol as solvent. The filtrate was evaporated to dryness in a rotary evaporator at 40 °C under reduced pressure and stored in a refrigerator at 2–8 °C for further use in experiments. The percentage yield of methanol extract of *F. dalhousiae* bark was found to be 2.73% w/w.

2.3. Experimental animals

Wistar albino rats (180–200 g) were used in this study. Animals were procured from Govt. veterinary animal house mannuthy, approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24–27 °C and humidity 60–65% with 12:12 light: dark cycles). Commercial pellet diet and water were provided *ad libitum*. The preliminary phytochemical analysis was carried out using the standard phytochemical methods of Harborne [19].

2.5. Acute toxicity study

The doses for the study were fixed based on Irwin test [20]. No mortality and behavioral alterations (physical signs of toxicity, such as writhing, gasping, palpitation and decreased respiratory rate) were observed in *F. dalhousiae* bark extract treated rats. There was no lethality or toxic reaction found at any selected dose until the end of the study.

2.6. Hypolipidemic activity in Triton WR-1339 induced rats

2.6.1. Induction of hyperlipidemia

This study was to examine the short-term effects of *F. dalhousiae* bark extract on Triton-induced hyperlipidemic rats. Wistar rats weighing between 180 and 200 g were used for the study. The hyperlipidemia was induced by the intraperitoneal injection of Triton WR-1339 (200 mg/kg) (Sigma Chemical Co., USA) dissolved in phosphate buffered saline (pH 7.4) [21]. The rats were starved overnight and divided into five groups of six rats each.

Group I served as normal control rats treated with vehicle; Group II hyperlipidemic rats were treated with the vehicle; Group III and IV hyperlipidemic rats were fed with high-fat diet and treated with the vehicle; Group II hyperlipidemic control rats were fed with high-fat diet and treated with the vehicle; Group III and IV hyperlipidemic rats were fed with high-fat diet and treated with *F. dalhousiae* extract at 250 and 500 mg/kg, respectively; Group V hyperlipidemic positive control rats were fed with high-fat diet and treated with Fenofibrate (65 mg/kg).

All the treatments were given orally, immediately after Triton injection. In the following period of study (18 h), rats had access only to water. After 18 h of treatment, the rats were euthanized and blood was collected. The blood samples were immediately centrifuged (2500 rpm/15 min) and the plasma was used for lipid analysis.

2.7. Effect of *F. dalhousiae* bark extract on high fat diet induced hyperlipidemic rats

2.7.1. Induction of hyperlipidemia

The long-term hypolipidemic effect of *F. dalhousiae* extract was studied in high fat diet fed-obese hyperlipidemic rat model. Male Wistar rats weighing 180–200 g were used in this study. The rats were fed with a high-fat diet composed of standard rat chow 68%, dalda (saturated fat) 30%, and cholesterol 2% for 15 days [22]. The rats were divided into five groups, with six rats in each group. Group I was normal control rats fed with normal pellet diet and treated with the vehicle; Group II hyperlipidemic control rats were fed with high-fat diet and treated with the vehicle; Group III and IV hyperlipidemic rats were fed with high-fat diet and treated with *F. dalhousiae* extract at 250 and 500 mg/kg, respectively; Group V hyperlipidemic positive control rats were fed with high-fat diet and treated with Fenofibrate (65 mg/kg).

Treatments were given once daily continuously for 28 days, orally. On days 0, 7, 14, 21, and 28, the blood samples was collected by retro orbital sinus and the TC, TG, HDL-C, and LDL-C levels were measured using diagnostic kits (Sigma Diagnostic, Inc., USA) [23]. At the end of the study, the rats were sacrificed, and blood and liver samples were collected. The liver samples were stored at −70 °C for the analysis of the TC and TG levels.

2.7.2. Biochemical analysis of serum

The TC, TG, and HDL-C levels were quantified using enzymatic kits (Sigma Diagnostic, Inc., USA). The LDL-C level was calculated using the formula of Friedewald et al. [24]: LDL-C = TC – (HDL-C + TG)/5, where TC is the total cholesterol level and TG is the triglycerides level.

2.8. Statistical analysis

The results were presented as mean ± SEM. Statistical analyses of all the data obtained were evaluated using one-way ANOVA followed by Student’s t-test (SPSS Program; Version 11.5). The differences were considered as significant at *P* < 0.05.

3. Results

3.1. Preliminary phytochemical analysis

The preliminary phytochemical evaluation of *F. dalhousiae* bark extract showed the presence of coumarins, flavonoids, steroids and tannins.

3.2. *F. dalhousiae* bark extract on Triton induced hyperlipidemia

The plasma TC, TG, LDL-C, and HDL-C levels of *F. dalhousiae* bark extract (250 and 500 mg/kg) treated groups were shown in Table 1. In comparison with the normal control group, Triton caused a marked increase on the plasma TC, TG, and LDL-C levels, and a decrease in the HDL-C level. After treatment with 250 and 500 mg/kg of the extract, *F. dalhousiae* bark extract (250 and 500 mg/kg) significantly reduced the levels of TC (*P* < 0.05 and *P* < 0.005), TG (*P* < 0.05 and *P* < 0.005), and LDL-C (*P* < 0.005).
**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>70.31 ± 2.81</td>
<td>103.73 ± 2.73</td>
<td>60.18 ± 4.29</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>Triton</td>
<td>250.18 ± 3.18</td>
<td>652.81 ± 2.91</td>
<td>205.11 ± 6.31</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>Triton + ME (250 mg/kg)</td>
<td>243.76 ± 2.31*</td>
<td>631.71 ± 1.21**</td>
<td>193.81 ± 2.73*</td>
<td>0.36 ± 0.03*</td>
</tr>
<tr>
<td>Triton + ME (500 mg/kg)</td>
<td>220.31 ± 2.89**</td>
<td>502.2 ± 2.41**</td>
<td>162.17 ± 3.81*</td>
<td>0.42 ± 0.09**</td>
</tr>
<tr>
<td>Triton + F (65 mg/kg)</td>
<td>140.23 ± 4.81**</td>
<td>262.73 ± 2.81**</td>
<td>73.81 ± 2.85**</td>
<td>0.59 ± 0.02**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 6). *P < 0.05, **P < 0.005, compared with the hypolipidemic control values.

and P ≤ 0.005), and increased the HDL-C (P ≤ 0.005 and P ≤ 0.005) levels.

### 3.3. F. dalhousiae bark extract on high fat diet induced hyperlipidemia

The plasma TC, TG, LDL-C and HDL-C levels of *F. dalhousiae* bark extract (250 and 500 mg/kg) treated groups on day 0, 7, 14, 21, and 28 were shown in **Table 2**. Normal rats fed with high-fat diet for 15 days showed increased plasma TC, TG, and LDL-C, and decreased HDL-C levels. After treatment with *F. dalhousiae* bark extract for 28 days, the lipid levels of 250 and 500 mg/kg treated animals (P ≤ 0.05 and P ≤ 0.005) significantly altered the levels near to normal.

The levels of liver TC and TG of normal and hyperlipidemic rats are shown in **Fig. 1**. The normal rats fed with high-fat diet for 15 days showed a significant increase (P ≤ 0.005) in the TC and TG levels of the liver. After treatment with methanol extract (250 and 500 mg/kg), the TC and TG levels of the liver were reduced to normal levels.

### 4. Discussion

Triton WR-1339 a non-ionic detergent inhibits the catabolising enzymes lipoprotein lipase (LPL) and lecithin cholesterol acetyl transferase (LCAT) by blocking the uptake of lipoprotein from the circulation by extra hepatic tissues, which cause an increase in the level of circulatory lipoproteins. Triton WR-1339 detergent incorporates free cholesterol into HDL and transferring back to VLDL or IDL, which is taken back by the liver cells. Several studies show that an increase in HDL cholesterol is associated with a decrease in coronary risk [26] and most of the drugs that decrease total cholesterol also decrease HDL cholesterol [27]. But in the present study, the extract of *F. dalhousiae* decreased the total cholesterol and LDL cholesterol and enhanced the HDL cholesterol significantly. This is an important advantage in treatment of hypercholesterolemia especially among Indians where low HDL cholesterol is the prevalent lipoprotein abnormality. LDL cholesterol is major coronary risk factors for atherosclerosis [28,29]. LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries, a rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence it is bad for health and a direct risk factor for coronary heart disease [30,31]. Administration of *F. dalhousiae* lowered both total and LDL cholesterol in experimental rats. The lowering of TC and LDL in serum by *F. dalhousiae* would reduce the incidence of coronary events. The decrease of serum TG level is an important finding of this experiment. Recent studies also showed that triglycerides are independently related to coronary heart disease [32,33] and most of the

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>0 day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>NC</td>
<td>76.48 ± 7.48</td>
<td>77.64 ± 8.37</td>
<td>79.37 ± 2.37</td>
<td>76.37 ± 4.37</td>
<td>77.38 ± 3.28</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>145.83 ± 2.38</td>
<td>157.87 ± 6.38</td>
<td>162.45 ± 7.38</td>
<td>169.36 ± 2.37</td>
<td>178.36 ± 5.38</td>
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<tr>
<td></td>
<td>HC + ME (250 mg/kg)</td>
<td>142.38 ± 2.37</td>
<td>130.58 ± 5.28*</td>
<td>120.62 ± 3.88**</td>
<td>109.34 ± 4.38**</td>
<td>101.52 ± 5.09**</td>
</tr>
<tr>
<td></td>
<td>HC + ME (500 mg/kg)</td>
<td>144.38 ± 3.58</td>
<td>140.38 ± 1.66*</td>
<td>101.81 ± 9.52*</td>
<td>92.12 ± 2.11**</td>
<td>82.45 ± 3.19**</td>
</tr>
<tr>
<td></td>
<td>HC + F (65 mg/kg)</td>
<td>147.26 ± 4.32</td>
<td>120.53 ± 6.18*</td>
<td>94.24 ± 1.58**</td>
<td>87.23 ± 2.41**</td>
<td>80.18 ± 1.09**</td>
</tr>
<tr>
<td>TG</td>
<td>NC</td>
<td>97.26 ± 3.81</td>
<td>99.28 ± 7.31</td>
<td>100.16 ± 4.83</td>
<td>99.22 ± 3.92</td>
<td>99.38 ± 4.84</td>
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<tr>
<td></td>
<td>HC</td>
<td>206.25 ± 4.79</td>
<td>245.63 ± 5.37</td>
<td>253.18 ± 4.27</td>
<td>259.78 ± 2.68</td>
<td>261.21 ± 5.24</td>
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<tr>
<td></td>
<td>HC + ME (250 mg/kg)</td>
<td>221.14 ± 3.25</td>
<td>227.24 ± 4.68</td>
<td>221.48 ± 2.38**</td>
<td>190.22 ± 4.18**</td>
<td>181.31 ± 3.19**</td>
</tr>
<tr>
<td></td>
<td>HC + ME (500 mg/kg)</td>
<td>242.36 ± 6.33</td>
<td>223.18 ± 4.28*</td>
<td>202.58 ± 7.97**</td>
<td>194.34 ± 7.14**</td>
<td>180.65 ± 6.89**</td>
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<tr>
<td></td>
<td>HC + F (65 mg/kg)</td>
<td>223.18 ± 6.33</td>
<td>197.38 ± 5.68**</td>
<td>183.75 ± 6.48**</td>
<td>163.78 ± 6.47**</td>
<td>143.62 ± 8.39**</td>
</tr>
<tr>
<td>LDL-C</td>
<td>NC</td>
<td>37.63 ± 3.28</td>
<td>41.78 ± 9.18</td>
<td>40.08 ± 2.35</td>
<td>40.18 ± 2.38</td>
<td>41.30 ± 2.21</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>93.24 ± 4.47</td>
<td>110.95 ± 6.58</td>
<td>115.16 ± 8.59</td>
<td>126.43 ± 3.39</td>
<td>130.61 ± 3.74</td>
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<td></td>
<td>HC + ME (250 mg/kg)</td>
<td>125.53 ± 2.67</td>
<td>120.83 ± 2.89</td>
<td>112.83 ± 7.42</td>
<td>115.36 ± 2.89*</td>
<td>110.31 ± 2.81**</td>
</tr>
<tr>
<td></td>
<td>HC + ME (500 mg/kg)</td>
<td>120.89 ± 2.43</td>
<td>110.34 ± 2.43</td>
<td>109.31 ± 2.82</td>
<td>104.31 ± 2.21**</td>
<td>97.83 ± 4.54**</td>
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<tr>
<td></td>
<td>HC + F (65 mg/kg)</td>
<td>126.68 ± 1.72</td>
<td>110.32 ± 2.18*</td>
<td>92.58 ± 9.40**</td>
<td>86.78 ± 5.32**</td>
<td>75.78 ± 5.21**</td>
</tr>
<tr>
<td>HDL-C</td>
<td>NC</td>
<td>37.23 ± 3.39</td>
<td>36.86 ± 9.37</td>
<td>35.52 ± 4.89</td>
<td>35.74 ± 2.62</td>
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<tr>
<td></td>
<td>HC</td>
<td>32.10 ± 5.25</td>
<td>28.25 ± 4.20</td>
<td>27.42 ± 5.20</td>
<td>26.20 ± 2.01</td>
<td>25.08 ± 1.19</td>
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<td>HC + ME (250 mg/kg)</td>
<td>34.78 ± 7.89</td>
<td>35.28 ± 3.50</td>
<td>35.67 ± 4.19</td>
<td>40.44 ± 3.53**</td>
<td>43.38 ± 5.20**</td>
</tr>
<tr>
<td></td>
<td>HC + ME (500 mg/kg)</td>
<td>34.62 ± 3.31</td>
<td>37.42 ± 2.29</td>
<td>39.85 ± 4.20</td>
<td>40.31 ± 2.19**</td>
<td>42.21 ± 2.11**</td>
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<tr>
<td></td>
<td>HC + F (65 mg/kg)</td>
<td>35.30 ± 4.03</td>
<td>41.62 ± 5.21</td>
<td>48.42 ± 4.37**</td>
<td>52.26 ± 3.33*</td>
<td>56.37 ± 10.28**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 6). *P ≤ 0.05, **P ≤ 0.005, compared with the hypolipidemic control values. NC-normal control, HD-hyperlipidemic control, ME-methanol extract, F-Fenofibrate.
antihypercholesterolemic drugs do not decrease triglycerides levels, but F. dalhousiae lowered it significantly and this effect might be related to increase the endothelium bound lipoprotein lipase which hydrolyses the triglycerides into fatty acids. The presence of ficusin have been reported in ficus species [34]. Ficusin showed antihyperlipidemic effect in high fat diet – streptozotocin induced diabetic rats [35]. The presence of this compound in F. dalhousiae may responsible for the antihyperlipidemic effect.

5. Conclusion

From the study, it has been proved that the methanol extract of F. dalhousiae stem bark has antihyperlipidemic effect in triton WR-1339 and high fat diet-induced hyperlipidemic rats. Further studies on isolation and identification of the active molecule will provide a new drug for clinical use.

Conflict of interest

None declared.

Acknowledgments

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References


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