Anti-hyperglycemic and Anti-hyperlipidemic Effects of the Triterpenoid-Rich Fractions from *Rubus coreanus* and *Rubus c. crataegifolius* and Their Main Component, Niga-ichigoside F₁, in Streptozotocin-induced Diabetic Rats

Jongwon Choi¹, Yeong-Min Yoo², Min-Young Kim², Jung-Hwan Nam³, Agung Nugroho², and Hee-Juhn Park²*  

¹College of Pharmacy, Kyungsung University, Busan 608-736, Korea  
²Department of Pharmaceutical Engineering, Sangji University, Wonju 220-702, Korea  
³Highland Agriculture Institute, Rural Development Administration, Pyeongchang 232-950, Korea

**Abstract** – To find the antidiabetic activity of the triterpenoid-rich fractions of *R. coreanus* (TRF-cor) and *R. c. crataegifolius* (TRF-cra) leaves or its main component niga-ichigoside F₁ (Niga-F₁), anti-hyperglycemic and anti-hyperlipidemic effects were investigated in the diabetic rat model induced by streptozotocin (STZ). Treatments of rats with 200 mg/kg of the TRF-cor, TRF-cra (each, p.o.) or 20 mg/kg of Niga-F₁ significantly inhibited the increase of blood glucose concentration about 44.8%, 28.7% or 20.6%, respectively, in the diabetic rats. In addition, treatments with those fractions inhibited the increase of serum concentrations of triglyceride, total cholesterol or LDL-cholesterol caused by STZ. The inhibitory rate on atherogenic index (AI) values of the TRF-cor (200 mg/kg), TRF-cra (200 mg/kg) or Niga-F₁ (20 mg/kg)-treated groups were decreased about 55.7%, 36.3% or 22.6%, respectively, comparable to STZ-treated group. In the oral glucose tolerance test, treatment of TRF-cor or TRF-cra inhibited the increase of blood glucose concentration in the STZ-induced rats. Administration of 20 mg/kg of Niga-F₁ (p.o.) also exhibited similar effects with the effects of both TRFs at 200 mg/kg dose (p.o.). These results support that the triterpenoids, in particular Niga-F₁, are contributed to the antidiabetic effects of *R. coreanus* or *R. c. crataegifolius*.

**Keywords** – *Rubus coreanus*, *R. c. crataegifolius*, triterpenoid, niga-ichigoside F₁, anti-hyperglycemic, anti-hyperlipidemic

---

**Introduction**

*R. coreanus* (Rosaceae) is a woody plant that grows wild in Korea. Its ripe fruit is an edible food, whereas the unripe fruit (Rubi Fructus) is used as an oriental herb medicine to treat diabetes mellitus and sexual impotency (Moon, 1994). We previously reported that Rubi Fructus had anti-nociceptive and anti-inflammatory effects (Choi et al., 2003), also anti-rheumatic and gastroprotective effects (Nam et al., 2006).

*R. coreanus* fruit turns black when ripe, while those of *R. c. crataegifolius* become red. The *R. coreanus* and *R. c. crataegifolius* fruits growing wild in Korea are applicable to black and red raspberries, respectively, in the western countries. Unripe *R. coreanus* fruit contains 19a-hydroxy-yursane-type triterpenoids, as does ripe *R. c. crataegifolius*. The leaves of these plants also contain large amounts of these triterpenoids (Nam et al., 2007).

*Author for correspondence*  
Tel: +82-33-730-0564; E-mail: hjpark@sangji.ac.kr

---

Diabetes mellitus, obesity, and atherosclerosis are increasing in developed countries, mainly due to changes in the diet. Diets rich in vegetables and functional foods as well as restriction of high calorie foods, can help treat or to prevent diabetes mellitus or hyperlipidemia. In this work, we elucidated the anti-diabetic effects of both the triterpenoid-rich fraction (TRF) of the leaves of *R. coreanus* and *R. c. crataegifolius* or its main component niga-ichigoside F₁ (Fig. 1) in streptozotocin (STZ)-induced rats by measuring body weight gain and food intake, blood glucose levels, levels of serum cholesterol and glucose tolerance.

**Materials and Methods**

**Plant material**– The leaves of *R. coreanus* and *R. c. crataegifolius* were collected in June near Wonju, Kangwon-Do, dried, and crushed for extraction.

**Preparation of TRF from *R. coreanus* and *R. c. crataegifolius*** – Plant material (500 g) was extracted with
Isolation of Niga-F₁—The TRF-cor (15 g) was chromatographed on a silica gel column (280 g, 5 × 55 cm) using CHCl₃-MeOH (4:1) as an eluent. The retention volumes of 0.90 - 1.20 L, 1.4 - 1.75 L, and 2.2 - 2.5 L were concentrated to dryness in vacuo to give Cor-Fr.1 (1.1 g), Cor-Fr.2 (2.9 g), and Cor-Fr.3 (4.2 g), respectively. The Cor-Fr.3 fraction was purified using silica gel column chromatography with CHCl₃-MeOH- H₂O (75:25:10, lower phase) to yield Niga-F₁. TRF-cra was also purified by the same chromatography steps to yield Niga-F₁ (Nam et al., 2006).

Niga-F₁—Amorphous powder from MeOH, mp, 233 - 6 °C, [α]D° +11.2° (c = 0.021, MeOH); ¹H- NMR and ¹³C-NMR: Literature (Nam et al., 2006).

Experimental animals—Male Sprague-Dawley rats were purchased from Daehan Bio Link Co., allowed to adapt to laboratory conditions (temperature: 20 ± 2 °C, dampness: 40 - 60%, light/dark cycle: 12 h) for a week, and rats weighing 200 ± 10 g were used for animal experiments. 24 h before the experiment, only water was offered to the animals. Considering variations in enzyme activity during one day, the animals were sacrificed at a fixed time (10:00 A.M. - 12:00 P.M.). These experiments were approved by the University of Kyungsung Animal Care and Use Committee. All procedures were conducted in accordance with the “Guide for Care and Use of Laboratory Animals” published by the National Institutes of Health.

Induction of experimental diabetes—Diabetes mellitus was induced by intravenous injection of STZ in the tail vein (Sigma, St. Louis, MO) dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) at 50 mg/kg body weight. After 48 h, only rats with fasting blood glucose levels 300 - 400 mg/dl were considered diabetic and used in the study.

Experimental design—Untreated (normal), diabetic (control), and TRF-treated rats were randomly assigned to 3 different groups (n = 9). One group received distilled water, a second group (control) had diabetes mellitus induced by STZ treatment, and the third STZ-treated groups received TRF-cor (100, 200 mg/ml), TRF-cra (100, 200 mg/ml), or Niga-F₁ (10, 20 mg/ml) for consecutive 4 weeks. The rats were treated orally. Rats were fasted 7 h after the final sample treatment, anesthetized with CO₂, and blood was collected from abdominal aortas. Serum was separated by centrifugation at 2,500 × g for 15 min.

Measurement of blood glucose concentrations, body weight, and food intake—Blood glucose concentration was measured by the method of Shirwaikar et al. (2006). Body weights were measured 24 h before STZ treatment and 24 h after sample treatment to calculate the change of body weights. Food intake was measured one day before sacrifice.

Oral glucose tolerance test—The STZ-induced diabetic rats were orally administered TRF-cor, TRF-cra and Niga-F₁, fasted for about 10 - 14 h, treated orally with 1.5 g/kg of glucose, and blood glucose concentration measured at 0, 30, 60 and 120 min.

Measurement of total cholesterol levels—Total serum cholesterol levels were measured by following the manufacturer’s protocol with an AM 202-K assay kit (Asan Pharm. Co., Korea) prepared by Richmond (1976). Three milliliter of solution (cholesterol esterase 20.5 U/l, cholesterol oxidase 10.7 U/l, sodium hydroxide 1.81/l, potassium phosphate monobasic 13.6 g/l, phenol 1.88 g/l) was mixed with 20 µl of serum and incubated at 37 °C for 5 min. For determination of total cholesterol levels, the absorbance of the reaction mixture was measured at
550 nm and levels were calculated using a standard calibration curve.

**Measurement of triglyceride levels** – Triglyceride levels were measured by following the manufacturer’s protocol with an AM 157S-K assay kit (Asan Pharm. Co., Korea) prepared by McGowan et al. (1983). Three milliliter of solution [lipoprotein lipase 10,800 U, glycerol kinase 5.4 U, peroxidase 135,000 U, L-α-glycerophosphoxidase 160 U, N,N-bis(2-hydroxyethyl)-2-aminomethylsulfonic acid 0.427 g/dl] was mixed with 20 µL of serum and incubated at 37°C for 10 min. For determination of serum triglyceride levels, the absorbance of the reaction mixture was measured at 550 nm and levels were calculated using a standard calibration curve.

**Measurement of high density lipoprotein-cholesterol (HDL) levels** – Serum HDL-cholesterol levels were measured by following the manufacturer’s protocol with an AM 203-K assay kit (Asan Pharm. Co., Korea) prepared by Noma et al. (1978). Twenty microliter of serum was mixed with two-hundred microliter of solution (dextransulfate 0.1%, magnesium chloride 0.1 M), incubated at room temperature for 10 min, and centrifuged at 2,500 × g for 10 min. The 100 µL of supernatant was mixed with 3 mL of solution (lipoprotein lipase 10,800 U, glycerol kinase 5.4 U, peroxidase 135,000 U, L-α-glycerophosphoxidase 160 U, N,N-bis(2-hydroxyethyl)-2-aminomethylsulfonic acid 0.427 g/dl) and incubated at 37°C for 5 min. For determination of serum HDL levels, the absorbance of the reaction mixture was measured at 500 nm and their levels were calculated as mg/dl using a standard calibration curve.

**Calculation of low density lipoprotein-cholesterol (LDL) levels** – LDL levels were calculated by following the Friedewald’s equation (Friedewald et al., 1972). LDL-cholesterol = total cholesterol - HDL-cholesterol - triglyceride/(5).

**Statistical analysis** – Results are expressed as means ± S.D. (n = 9). Statistical analysis was performed with Duncan’s multiple range tests. Differences were considered significant at p < 0.05.

**Results and Discussion**

The STZ-treated group (control group) showed increased blood glucose concentrations, with TRF-cor and TRF-cra (both at 200 mg/kg) or Niga-F1 (20 mg/kg) suppressing these increases by 44.8%, 28.7% and 20.6%, respectively (Fig. 2). These results suggest that the triterpenoids of *R. coreanus* or *R. crataegifolius* or their main component, Niga-F1, may be effective in treating diabetes mellitus.

The atherogenic index (AI) is a measure of hyperlipidemia that incorporates: 1) increased chylomicron biosynthesis in the small intestine; 2) increased triglyceride,
LDL- and VLDL-cholesterol biosynthesis in the liver; 3) decreased HDL-cholesterol biosynthesis in the liver; 4) decreased lipase activity and removal of triglycerides in peripheral tissue (Miller, 1978). Hyperlipidemia often leads to atherosclerosis. In addition, lipid peroxides and oxygen free radicals produced from LDL peroxidation are toxic to endothelial cells. Oxidized LDLs are adhesive to blood vessel walls, leading to further accumulation of other oxidized LDLs, platelets, and macrophage cells to the vessel walls (Steinberg, 1997). STZ treatment increased serum triglyceride, total- and LDL-cholesterol, but did not markedly change HDL-cholesterol, leading to an increased AI value compared to the untreated group. TRF treatment decreased the hyperlipidemia and high AI caused by STZ treatment. AI values of the STZ-treated group, TRF-cor (200 mg/kg dose) or TRF-cra (200 mg/kg dose)-treated groups were $2.53 \pm 0.48$, $1.12 \pm 0.03$, or $1.61 \pm 0.17$, respectively, while that of the Niga-F$_1$ (20 mg/kg dose) group was $1.96 \pm 0.15$. AI inhibition by TRF-cor, TRF-cra and Niga-F$_1$ were $55.7\%$, $36.3\%$, and $22.6\%$, respectively (Fig. 3). These results indicate that the triterpenoid in R. coreanus or R. crataegifolius and its main component, Niga-F$_1$, inhibits hyperlipidemia and atherosclerosis due to diabetes mellitus. This anti-hyperlipidemic effect, particularly decreasing the AI value, was stronger than the anti-hyperglycemic effect. The activity of TRF-cor was more potent than that of TRF-cra.

After oral administration of glucose (1.5 g/kg) to STZ-treated rats, blood glucose concentrations were measured from 0 to 120 min. Blood glucose concentrations in the STZ-treated group increased up to 120 min, indicating reduced glucose tolerance (Fig. 4). Treatment with TRF-cor (200 mg/kg), TRF-cra (200 mg/kg), or Niga-F$_1$ (20 mg/kg) inhibited the reduced glucose tolerance by STZ treatment, indicating that they contributed to blood glucose transport into cells. TRF-cor had more potent anti-hyperglycemic and anti-hyperlipidemic activities than TRF-cra.

### Table 2. Effect of TRF-cor, TRF-cra, or Niga-F$_1$ on the concentration of triglyceride, total cholesterol (total), HDL-cholesterol, LDL-cholesterol (LDL), or atherogenic index (AI) in STZ-treated hyperglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Triglyceride (mg/dl)</th>
<th>Total (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>74.3 ± 6.25$^d$</td>
<td>64.8 ± 5.49$^e$</td>
<td>38.9 ± 4.17$^e$</td>
<td>12.1 ± 2.42$^f$</td>
</tr>
<tr>
<td>STZ</td>
<td></td>
<td>140.8 ± 9.27$^a$</td>
<td>151.3 ± 4.57$^b$</td>
<td>42.9 ± 2.49$^{a,c}$</td>
<td>80.3 ± 0.84$^d$</td>
</tr>
<tr>
<td>TRF-cor</td>
<td>100</td>
<td>120.4 ± 5.69$^{d,h}$</td>
<td>136.4 ± 4.53$^b$</td>
<td>45.3 ± 3.17$^b$</td>
<td>65.1 ± 4.25$^c$</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>96.8 ± 3.11$^{a,f}$</td>
<td>108.7 ± 5.17$^c$</td>
<td>51.4 ± 2.13$^c$</td>
<td>39.7 ± 3.97$^c$</td>
</tr>
<tr>
<td>TRF-cra</td>
<td>100</td>
<td>128.8 ± 7.28$^{c,d}$</td>
<td>144.6 ± 4.25$^{a,b}$</td>
<td>43.8 ± 3.09$^{c,d}$</td>
<td>74.3 ± 5.11$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>103.8 ± 6.25$^{c,f}$</td>
<td>123.9 ± 4.86$^{a,c}$</td>
<td>47.6 ± 3.11$^{a,b}$</td>
<td>53.5 ± 4.18$^d$</td>
</tr>
<tr>
<td>Niga-F$_1$</td>
<td>10</td>
<td>135.2 ± 6.43$^{c,d}$</td>
<td>150.9 ± 3.45$^c$</td>
<td>43.1 ± 2.25$^{a,b}$</td>
<td>79.8 ± 4.25$^c$</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>112.4 ± 8.20$^{a,e}$</td>
<td>137.9 ± 4.21$^b$</td>
<td>46.5 ± 2.10$^{a,b}$</td>
<td>68.9 ± 3.23$^{a,b}$</td>
</tr>
</tbody>
</table>

The assay procedures are described in the materials and methods. Data represents means ± S.D. (n = 9). Values followed by the same superscript letter are not significantly different from each other (p < 0.05) by Duncan’s multiple range test. LDL-Cholesterol: Total Cholesterol – (HDL-Cholesterol + Triglyceride/5); Atherogenic Index (AI): (Total Cholesterol – HDL-Cholesterol)/HDL-Cholesterol.
In conclusion, the triterpenoids of *R. coreanus* or *R. crataegifolius* contribute to the traditional therapeutic use in treating diabetes mellitus, with Niga-F1 as a key component. Also, TRF-cor has greater potency than TRF-cra in animal models. The two TRFs exhibited more potent inhibitory effects on hyperlipidemia than on hyperglycemia.

Acknowledgements

We gratefully acknowledge the financial support provided by the Rural Development Administration of the Korean Ministry of Agriculture and Forestry.

References


(Accepted December 15, 2008)