Effects of Aster scaber Seed Oil Containing trans-Δ3 Fatty Acids on Lipid Profiles of Hamsters and Rats

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Abstract: The effects of Aster scaber seed oil (ASO) on lipid profiles were studied in rats and hamsters. ASO contained considerable amounts of Δ3t-16:1 (11.4%), Δ3t, 9c-18:2 (4.6%), and Δ3t, 9c, 12c-18:3 (11.3%). Young rats and hamsters were fed diets containing ASO, soybean oil (SBO), or olive oil (OLO) as fat sources for 4 weeks in separate experiments with or without cholesterol. In the rat study, there were no significant differences in the concentrations of serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triacylglycerol among the groups. The serum but not liver malondialdehyde (MDA) level was significantly lower in the ASO-fed group than it was in the other groups. The biochemical and growth parameters revealed no significant biological damages in the ASO-fed animals. In the hamster study, dietary cholesterol-dependent effects were evident in the serum lipids profiles, whereas the fat-induced effect was only observed in the ratio of serum low-density lipoprotein (LDL)-/HDL-cholesterol. Furthermore, fat- and cholesterol-induced effects were evident in the ratio of serum LDL-/HDL-cholesterol. Significant interactions between dietary fat and cholesterol were observed as evident from the concentration of serum cholesterol and triacylglycerol, as well as the activity of serum cholesterol ester transfer protein. These results suggest that dietary ASO containing trans-Δ3 fatty acids appeared to improve the serum LDL-/HDL-cholesterol ratio more than the SBO did, especially when hamsters were simultaneously fed cholesterol-supplemented diet.

Key words: Aster scaber seed oil, cholesterol, trans-Δ3 fatty acids, hamsters, rats

1 INTRODUCTION

The leaves of Aster scaber Thunb. are an edible wild vegetable that is popular in Korea. It is mainly used in salads with meat, seasoned greens, and other parboiled vegetables¹. Interestingly, the seed oil of A. scaber contains unusual trans-Δ3-enolic acids namely tΔ3-16:1; tΔ3, cΔ9-18:2 and tΔ3, cΔ9, 12-18:3 while the seed oil of Chrysanthemum zawadskii Herb. contains cΔ3, cΔ9, 12-18:3.¹,² The presence of peculiar fatty acids in plants appears to provide clues to the relationship between plant species, genera, or families.³,⁴

In this context, we and others have reported the effects of unfamiliar Δ3-fatty acids such as columbinic acid(trans-Δ5,9,12-octadecatrienoic acid), juniperonic acid(all cis-Δ5,11,14,17-octadecatetraenoic acid), pinolenic acid(all cis-Δ5,9,12-octadecatrienoic acid), and sciadonic acids(all cis-Δ5,11,14-octadecatrienoic acid) from seed oils on lipid metabolism in mice and rats.⁵-⁸ These observations demonstrated that some geometric differences in dietary fatty acids affected lipid metabolism in animals. To date, no evidence of the effects of trans-Δ3 fatty acids on lipid metabolism has been reported.

Therefore, in the present study, we examined the effects of dietary A. scaber seed oil (ASO), which is rich in trans-Δ3 fatty acids, on the lipid profiles of rats and hamsters. Rodents such as rats and hamsters have been used to evaluate the effects of novel compounds on blood lipids profiles. In particular, the cholesterol metabolism of hamsters is different from that of rats and is similar to that of humans.⁹,¹⁰

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2 EXPERIMENTAL PROCEDURES

2.1 Materials
The seeds of *A. scaber* were collected from a farm located in Kapyeong, Kyeonggi province, Korea, in October 2012 and then they were ground and extracted with hexane thrice. The oil was obtained from the seed extracts, collected by evaporation under nitrogen gas, and then stored at −30°C in a refrigerator. In order to evaluate the net effect of ASO, we used the crude oil in this preliminary experiment. The other dietary oils used were obtained commercially.

2.2 Treatment of animals
We conducted two experiments using rats and hamsters in experiments 1 and 2, respectively. Five-week-old Sprague-Dawley rats and golden Syrian hamsters were purchased from Charles River Japan (Yokohama, Japan). The animals were fed with commercial pellets freely for 2 weeks. They were housed individually in a temperature-controlled room (23-25°C) with a 12-h light/dark cycle. The diets were basically prepared following the AIN-93G purified diet recipe\(^\text{[1]}\). The animal study was approved by the Review Board of The Animal Ethics Committee of the Kangwon National University (KIAUC-09-0032), and we followed the University Guidelines for the Care and Use of Laboratory Animals.

In experiment 1, rats were randomly divided to three groups according to the different dietary fat sources. The diet comprised casein (20.0%), cornstarch (39.7%), pregelatinized corn starch (13.2%), dietary oil (7.0%), cellulose powder (5.0%), mineral mixture (AIN-93G-MX, 3.5%), vitamin mixture (AIN-93-VX, 1.0%), L-cysteine (0.3%), choline bitartrate (0.25%), tert-butylhydroquinone (0.0014%), sucrose (9.88%), and cholesterol (0.12%). In addition, soybean oil (SBO), olive oil (OLO), or ASO were used as dietary oils (Table 1). In experiment 2 with the hamsters, the diet was composed of casein (20.0%), cornstarch (39.7%), pregelatinized corn starch (13.2%), dietary oil (7.0%), cellulose powder (5.0%), mineral mixture (AIN-93G-MX, 3.5%), vitamin mixture (AIN-93-VX, 1.0%), L-cysteine (0.3%), choline bitartrate (0.25%), tert-butylhydroquinone (0.0014%), and sucrose (9.5%). As a dietary fat source, the same batches of SBO and ASO used in experiment 1 were used as dietary fat sources for the hamsters as well. Moreover, the diets were supplemented with or without cholesterol (0.5%) at the expense of sucrose in order to determine the effects of dietary cholesterol. In both experiments, the animals were allowed free access to the experimental diets for 4 weeks while food intake and body weight were measured every other day.

2.3 Biochemical analysis
At the end of the feeding period, the animals were fasted for 12 h and then euthanized by decapitation in both experiments. The organs including the livers were excised, washed, and stored at −70°C until analyzed. Blood was collected in sterile test tubes, allowed to clot, and centrifuged at 1,500 × g for 15 min to obtain the serum.

Commercially available kits were used to measure the concentration of serum total cholesterol, triacylglycerol (Asan Pharmaceutical Co., Ltd., Seoul, Korea), high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol (Daiichi Pure Chemical Industries, Co., Ltd., Tokyo, Japan), and nonesterified fatty acids (NEFA, Wako Pure Chemical Industries, Co., Ltd., Osaka, Japan). Serum cholesterol ester transfer protein (CETP) activity was determined using a CETP fluorometric assay kit II (Biovision Inc., CA, USA) following the manufacturer’s instructions. In addition, serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were assayed using a commercially available kit (Asan Pharmaceutical Co., Ltd., Seoul, Korea).

Liver lipids were extracted using the method of Folch et al.\(^\text{[2]}\). The lipid extracts were dissolved in acetone containing Triton X-100\(^\text{[3]}\). Liver cholesterol and triacylglycerol were enzymatically determined using the kits mentioned above. Lipid peroxide levels of the serum and liver were determined following the protocol previously described by Zhou et al.\(^\text{[4]}\). The protein content was estimated using the method of Lowry et al.\(^\text{[5]}\).

The fatty acid composition of the dietary oils was determined using a gas chromatography system equipped with a DB-23 column (60 m × 25 mm × 0.15 μm, J&W Scientific, CA, USA) as reported previously by Petrovic et al.\(^\text{[6]}\). For 12 experiments.

## Table 1  Fatty acid composition of dietary oils used in the experiments.

| Fatty Acid | SBO | OLO | ASO |
|-----------|-----|-----|-----|
| 16:0      | 12.1| 12.4| 4.8 |
| 3r-16:1   | 3.8 | 3.1 | 2.6 |
| 18:0      | 11.4| 11.4| 2.6 |
| 18:1 n-7  | 0.5 | 0.5 | 0.5 |
| 18:1 n-9  | 16.6| 16.6| 16.6|
| 3r, 9c-18:2| 4.6 | 4.6 | 4.6 |
| 18:2n-6   | 46.4| 46.4| 46.4|
| 3r, 9c, 12c-18:3| 11.3| 11.3| 11.3|
| 18:3 n-3  | 4.6 | 4.6 | 4.6 |
| 20:0      | 0.6 | 0.6 | 0.6 |

SBO, soybean oil; OLO, olive oil; ASO, *Aster scaber* seed oil.

2.4 Data analysis
Values are presented as mean ± standard error of the
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3 RESULTS

3.1 Experiment 1

There were no significant differences in the growth parameters, organ weight, or food intake between the groups (Tables 2 and 3). As shown in Table 4, the concentration of serum total cholesterol tended to be lower in rats fed the ASO diet than in those fed the other oils, although the trend was not statistically significant. No significant differences were found in the concentrations of serum HDL-cholesterol, triacylglycerol, NEFA, and MDA. However, the concentration of serum GOT and GPT tended to be lower in rats fed the ASO diet than in those fed the other oils.

Table 2 Growth parameters in experimental rats.

| Parameters                  | SBO       | OLO       | ASO       |
|-----------------------------|-----------|-----------|-----------|
| Initial body weight (g)     | 207 ± 2   | 207 ± 2   | 207 ± 1   |
| Final body weight (g)       | 361 ± 11  | 356 ± 13  | 354 ± 9   |
| Body weight gain (g/4 weeks)| 154 ± 9   | 149 ± 11  | 147 ± 8   |
| Food intake (g/day)         | 25.5 ± 1.2| 23.5 ± 1.1| 24.0 ± 0.2|
| FER                         | 0.26 ± 0.01| 0.27 ± 0.01| 0.28 ± 0.01|

Values are Mean ± SEM of 7 rats.
See the legend of Table 1.
FER, food efficiency ratio (total weight gain/total food intake).

Table 3 Weights of the various organs in experimental rats.

| Organs (g/100g body weight) | SBO       | OLO       | ASO       |
|-----------------------------|-----------|-----------|-----------|
| Liver                       | 3.40 ± 0.07| 3.37 ± 0.11| 3.35 ± 0.06|
| Spleen                      | 0.19 ± 0.01| 0.18 ± 0.01| 0.20 ± 0.01|
| Kidneys                     | 0.69 ± 0.02| 0.66 ± 0.01| 0.67 ± 0.01|
| Abdominal Fat-pad           | 2.45 ± 0.14| 2.37 ± 0.20| 2.46 ± 0.11|
| Epididymal Fat-pad          | 1.90 ± 0.20| 1.98 ± 0.17| 1.85 ± 0.10|

Values are Mean ± SEM of 7 rats.
See the legend of Table 1.

Table 4 Concentration of serum parameters in experimental rats.

| Parameters                  | SBO       | OLO       | ASO       |
|-----------------------------|-----------|-----------|-----------|
| Total cholesterol (mg/dL)   | 86.0 ± 5.4| 85.8 ± 6.0| 65.1 ± 6.2|
| HDL-cholesterol (mg/dL)     | 37.8 ± 3.1| 38.4 ± 2.9| 31.4 ± 3.2|
| HDL-chol/T-chol (%)         | 43.8 ± 1.5| 44.8 ± 1.0| 48.6 ± 2.5|
| Triacylglycerol (mg/dL)     | 123 ± 14  | 163 ± 18  | 114 ± 14  |
| NEFA (mmol/L)               | 0.71 ± 0.04| 0.66 ± 0.03| 0.65 ± 0.03|
| MDA (nmol/mL)               | 20.6 ± 2.6^b| 27.0 ± 1.0^b| 11.4 ± 2.8^a|
| GOT (IU/L)                  | 84.9 ± 10.1| 81.6 ± 5.3| 85.4 ± 9.1|
| GPT (IU/L)                  | 55.4 ± 14.4| 59.5 ± 2.9| 55.7 ± 2.9|

Values are Mean ± SEM of 7 rats.
See the legend of Table 1.
HDL-chol/T-chol, the ratio of HDL-cholesterol/total cholesterol; NEFA, non-esterified fatty acid; MDA, malondialdehydes; GOT, glutamine oxaloacetic transaminase; GPT, glutamic pyruvic transaminase.
Means not sharing a common superscript letter significantly differ at p < 0.05.
olesterol and triacylglycerol among the groups. The concentration of serum malondialdehyde (MDA) was significantly lower in the ASO-fed group than it was in the other groups. There were no differences in serum GOT and GPT activities among the groups; the activity levels of serum GOT and GPT were in the normal range.\(^{17}\)

The concentration of liver cholesterol tended to be lower in the SBO-fed group than it was in the OLO- and ASO-fed groups. The concentration of liver triacylglycerol and MDA were comparable among the groups (Table 5).

3.2 Experiments 2

Since SBO and OLO are composed of cis-form of fatty acids, we used ASO and SBO as dietary sources of fat in experiment 2 using hamsters. There were no significant differences in the growth parameters except in the food intake among the groups; fat-dependent effect was found in the food intake (Table 6).

Table 7 shows the concentration of serum lipids in hamsters. Significant cholesterol-dependent effects were observed in serum lipids, and interaction effects between dietary cholesterol and fat type were evident in the concentration of serum cholesterol and triacylglycerol. The

| Parameters                      | SBO (−) | ASO (−) | SBO (+) | ASO (+) | Fat (F) | Cholesterol (C) | F × C |
|---------------------------------|---------|---------|---------|---------|---------|-----------------|-------|
| Total cholesterol (mg/dL)       | 174 ± 7*| 168 ± 8*| 316 ± 12*| 349 ± 7*| NS      | p<0.05          | p<0.05|
| HDL-cholesterol (mg/dL)         | 106 ± 4 | 108 ± 6 | 168 ± 9 | 186 ± 4 | NS      | p<0.05          | NS    |
| LDL-cholesterol (mg/dL)         | 51.0 ± 3.3 | 51.8 ± 3.5 | 115 ± 4 | 107 ± 4 | NS      | p<0.05          | NS    |
| HLD-chol/T-chol (%)             | 61.3 ± 2.2 | 64.3 ± 2.2 | 53.1 ± 1.2 | 53.4 ± 1.6 | NS      | p<0.05          | NS    |
| LDL-chol/HDL-chol (%)           | 50.0 ± 5.2 | 48.0 ± 2.6 | 68.6 ± 1.3 | 57.3 ± 1.8 | p<0.05 | NS              | NS    |
| Triacylglycerol (mg/dL)         | 350 ± 39* | 235 ± 17* | 375 ± 37* | 407 ± 20* | NS      | p<0.05          | p<0.05|
| CETP activity (pmol/μL/hr)      | 27.9 ± 2.4* | 24.4 ± 1.6* | 40.9 ± 2.7* | 46.6 ± 1.4* | NS      | p<0.05          | p<0.05|

Values are Mean ± SEM of 8 hamsters. See the legend of Table 1 and Table 4.

LDL-chol/HDL-chol, the ratio of LDL-cholesterol/HDL-cholesterol; CETP, cholesteryl ester transfer protein.

Means not sharing a common superscript letter significantly differ at \( p < 0.05 \).
concentration of serum triacylglycerol was significantly lower in the ASO-fed group than it was in the SBO-fed group when the hamsters were fed the cholesterol-free diets. However, no difference was observed when the animals fed 0.5% cholesterol-supplemented diet. Furthermore, cholesterol supplementation significantly increased the concentration of serum total, HDL-, and LDL-cholesterol and decreased the ratio of serum HDL-cholesterol to total cholesterol. On the other hand, the ratio of LDL-/HDL-cholesterol increased significantly in the SBO- but not in the ASO-fed group. In addition, significant fat- and cholesterol-induced effects were observed in the ratio of serum LDL-/HDL-cholesterol. A cholesterol-induced effect was evident in the activity of serum CETP while there were significant interaction effects between fat types and cholesterol.

Table 8 shows the contents of liver cholesterol and triacylglycerol in hamsters. Furthermore, a significant cholesterol-induced effect was observed in the content of liver cholesterol but not in triacylglycerol level.

### Table 8  Concentration of liver parameters in experimental hamsters.

| Parameters                   | Cholesterol (-) | Cholesterol (+) | ANOVA |
|------------------------------|-----------------|-----------------|-------|
|                              | SBO             | ASO             |       |
| Total cholesterol (mg/g)     | 4.90 ± 0.30     | 5.41 ± 0.22     |       |
| Triacylglycerol (mg/g)       | 9.68 ± 1.03     | 9.18 ± 0.52     |       |

Values are Mean SEM of 8 hamsters.
See the legend of Table 1.

regarded as an index of atherogenicity. Hamsters have been shown to be similar in humans in serum lipoprotein, cholesterol, and bile metabolism, to some extent. These results suggest that the effect of dietary ASO containing trans-Δ3 fatty acids on serum cholesterol profiles varied between these animal species. Nevertheless, the ASO-dependent serum triacylglycerol-lowering effect was exclusively observed in hamsters fed with the cholesterol-free diets. Therefore, it is likely that the dietary cholesterol played a critical role in determining the serum lipids in hamsters fed with ASO.

The accumulated liver cholesterol, which occurred in the cholesterol-fed hamsters might have suppressed cholesterol synthesis and probably reduced the activity of the hepatic LDL receptor, leading to the increase in serum cholesterol levels. The reduction of the ratio of serum HDL-cholesterol to total cholesterol in response to cholesterol supplementation in hamsters might be partly related to the increased CETP activity under these experimental conditions. Recently, raising HDL-cholesterol, based on the inhibition of CETP activity, was introduced as a strategy to reduce cardiovascular risks.

The serum GOT and GPT activities were comparable between the ASO and SBO-fed animals. In addition, the stationary organ weights and favorable response of serum MDA levels to ASO feeding indicates that A. scaber seeds may be a potential source of healthy edible oil, which supports its historical use. Therefore, it is noteworthy that the dietary trans-Δ3 fatty acids decreased the serum lipid peroxidation, which is in agreement with the in vitro observations previously reported by Sargis and Subbaiah. It was also reported that trans-Δ3 16:1 and trans-Δ7 16:1 fatty acids in ruminant lipids, which were most likely derived from the animals’ feed, were present in cows, goats, and ewe cheese at a low level and exerted favorable health benefits. Nevertheless, the variation in feed intake due to fat sources should be considered as a possible factor that affected the lipids profiles observed in hamsters, although available data has shown that altering dietary fat sources and sucrose rather than variations in the feed intake, induced the changes in the lipid metabolism of rats.

To the best of our knowledge, this is the first report to describe the lipid-lowering effect of dietary ASO, and
further studies are needed to elucidate trans-Δ3 fatty acid metabolism including eicosanoids synthesis, as well as the detailed mechanism of the actions of ASO observed in this study.

5 CONCLUSION

ASO contains considerable amounts of Δ3t-16:1 (11.4%), Δ3t, 9c-18:2 (4.6%), and Δ3t, 9c, 12c-18:3 (11.3%). The effect of dietary ASO on serum lipid levels was varied in the animal species used in this study. Dietary ASO containing trans-Δ3 fatty acids appeared to markedly improve the ratio of serum LDL-/HDL-cholesterol compared to SBO, especially in hamsters fed cholesterol-supplemented diets.

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