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FATTY ACID AND AMINO ACID COMPOSITIONS OF *Artemisia sphaerocephala* SEED AND ITS INFLUENCE ON MOUSE HYPERLIPIDEMIA

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Artemisia sphaerocephala Krasch. (Asteraceae) is a perennial half-shrub, which is mainly distributed in desert areas in Gansu, Ningxia, and western Inner Mongolia, China [1, 2]. The surface of the seed is covered by a layer of gum, Artemisia seed polysaccharide [3]. *Artemisia* seed is edible, and the local people make noodles with it as a special ingredient. It is also a traditional Chinese herbal medicine, which is helpful in the therapy of some diseases, such as parotitis and abdominal distention, based on its function as a detumescent [4]. In addition, the seed is used as a folk medicine by diabetic patients [5].

Six fatty acids from *A. sphaerocephala* seed were determined by Bai et al. using GC [4], and the oil chemical composition was determined by Ma et al. with GC/MS [6]. It was found that *A. sphaerocephala* seed oil (ASSO) contains five fatty acids that accounted for 70% of the seed oil, including 65.70% α-linoleic acid. The content of vitamin E (VE) was much high and reached 1.17×10^3 mg/kg [6].

Abnormalities in lipid metabolism are the main reasons for hyperlipidemia, and hyperlipidemia potentially induces atherosclerosis. In particular, increase in triglyceride (TG), total cholesterol (TC), and low density lipoprotein-cholesterol (LDL-C) in serum is one of the factors for the atherosclerotic risk [7]. Therefore, a search for natural and nontoxic drugs is necessary for hyperlipidemia therapy. However, there are no extensive reports about the composition and stability of seed oil fatty acid and its influence on mouse hyperlipidemia-related indexes, such as serum TG, TC, and T-SOD levels. The aims of this paper are: 1) to determine the fatty acid composition and content of ASSO in the Alashan region of Inner Mongolia, China; 2) to evaluate the stability of seed oil under normal temperature and its effect on blood fat in mouse; 3) to investigate the amino acid content in the seed.

Table 1 shows the contents of 18 kinds of amino acids in *A. sphaerocephala* seed. The total amino acid content was 23.24%, which included 9.11% essential amino acids. The content of glutamic acid was the highest at 6.08%, followed by aspartic acid and arginine.

Analysis of ASSO obtained by Soxhlet extraction of fat is shown in Table 2. Using GC/MS, we identified ten compounds and four saturated fatty acids (7.81), which accounted for 99.92% of the total oil content, including six unsaturated fatty acids (92.18%) (Table 2).

The storage stability of fatty acids was evaluated (Table 2). After storage at 25°C for 150 days, there was no significant alteration in the contents of any other unsaturated fatty acids except for arachidonic acid content, which indicated that the oil can be stored for a long time. Compared with fresh oil, the unsaturated fatty acid content only decreased by 0.18%, which may be due to the high content of Vitamin E (Table 2). Vitamin E, a fat-soluble vitamin, is a major antioxidant. It is responsible for terminating free radical chain reactions that result from the oxidation of polyunsatured fatty acids (PUFA) [8, 9]. PUFA are considered essential fatty acids. Ibrahim et al. suggested that an increase in Vitamin E supplementation can inhibit cellular lipid peroxidation of PUFA [10].

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TABLE 1. *A. sphaerocephala* Seed Amino Acids, %

Essential amino acid	%	Nonessential amino acid	%
Thr	0.80	Asp	2.28
Val	0.81	Ser	1.11
Met	0.13	Glu	6.08
Ile	0.94	Gly	1.41
Leu	1.57	Ala	1.33
Phe	1.11	Tyr	0.59
Lys	0.99	Pro	0.79
His	0.55	Cys	0.54
Arg	1.91	Subtotal	14.13
Trp	0.30		
Subtotal	9.11		
Total	23.24		

TABLE 2. The Influence of Storage Time on *A. sphaerocephala* Seed Oil Chemical Composition at 25°C

Fatty acid	Fresh oil, %	150 days storage, %	Fatty acid	Fresh oil, %	150 days storage, %
Color	Canary yellow	Red brown	18:3	0.58	0.72
16:0	6.44	6.56	20:0	0.53	0.54
16:2	0.21	0.16	20:1	1.45	0.42
18:0	0.56	0.44	22:0	0.28	0.46
18:1	10.75	10.38	22:1	0.51	0.38
18:2	78.61	79.88	Total	99.92	99.94

TABLE 3. The Influence of *A. sphaerocephala* Seed Oil on Mouse Hyperlipidemia Indexes TG, TC Level, and T-SOD Activity ($\bar{x} \pm SD$)

Group	Does	TG, mmol/L	TC, mmol/L	T-SOD activity
Blank group	–	0.85 ± 0.49	2.06 ± 0.19	161.05 ± 3.83
Hyperlipidemia group	–	5.32 ± 0.96	3.22 ± 0.11	94.34 ± 16.88
Lovastatin group	0.05 mg/kg	1.28 ± 0.59	2.12 ± 0.69	52.89 ± 31.94
Low dose	0.10 mL/10 g	1.64 ± 1.27	3.12 ± 0.49	167.69 ± 26.78
Medium dose	0.15 mL/10 g	1.36 ± 1.12	2.80 ± 0.56	179.20 ± 33.26
High dose	0.20 mL/10 g	1.85±0.64	2.71 ± 0.26	178.44 ± 27.73

Lower-case letters within a column indicate differences at $P < 0.01$ level, capital letters indicate differences at $P < 0.05$ level.

The results of acute toxicity experiments show that ASSO is not harmful and could be taken safely. Ten mice were fed with the seed oil (5 mL/kg). After observation for two weeks, no toxic symptom was found in the mice. The hyperlipidemic mice model was also achieved successfully. After being fed with a high-fat diet for ten days, three mice were selected from each group to test the TG content in the serum. The content of TG (4.08 mmol/l) was 4.98 times higher than that in the blank group (0.82 mmol/l) ($P < 0.001$), which means that the hyperlipidemic mice model was achieved successfully. Moreover, after being fed for 40 days, the TG and TC contents of the hyperlipidemia group were 6.26 and 1.56 times higher than that in the blank group, respectively (Table 3). The index of T-SOD activity decreased by 41.42% ($P < 0.05$) as compared with the blank group. As shown in Table 3, after being fed with ASSO for 30 days, all these three different doses of ASSO given to hyperlipidemic mice can significantly decrease the level of TG in serum without an obvious dose-effect relationship, but has no significant effect on TC. Further studies are needed to examine the exact mechanism behind its effect. Additionally, the mouse TG contents in all these three ASSO groups were comparable to that in the lovastatin group.

All these three seed oil doses significantly increased mouse serum T-SOD activity (Table 3, $P < 0.01$). When lovastatin was taken, it not only significantly decreased the contents of TG and TC in hyperlipidemic mice, but also reduced the mouse serum T-SOD activity as compared with the blank group. *A. sphaerocephala* seed oil significantly increased the mouse serum T-SOD activity, possibly due to its high VE content. Shireen et al. [11] found that vitamins E and C separately or VE and VC in combination could increase AOE (antioxidant enzyme) activity in the liver, and also found that the combined use of vitamins C and E could significantly reduce the risk of chronic diseases related to oxidative stress.

Seeds of plant *A. sphaerocephala* were collected from Alashan Zuoqi of Inner Mongolia, China.

Extraction and Esterification of Seed Oil. Forty grams *A. sphaerocephala* seed was triturated and refluxed three to four times with petroleum ether (30–60°C). To the yellow oil obtained from the extraction, we added 4.5 g KOH and 200 mL ethanol into the oil, then refluxed it with water for 3 h and evaporated the ethanol under reduced pressure. We added 50 mL of 50% (v %) ether/water into the oil and isolated the ether layer after vibrating. The water layer was extracted with ether three times and combined with the ether. We controlled the pH of the water layer at 2–3 with 15% HCl and washed the ether layer with distilled water to pH 5–6. The ether layer was dried with anhydrous Na_2SO_4 overnight. The ether was evaporated under reduced pressure to get the total fatty acids. The fatty acids (1.0 g) were put into round-bottom flask with 5 mL methanol and 1.5 mL 120% BF_3/ether. We refluxed it for 20 min with water and evaporated the methanol under reduced pressure. Then we added 2.5 mL H_2O and 5.0 mL ether, vibrated the flask, then separated the ether layer. We extracted the water layer with ether three times and combined the ether extracts. We washed the ether layer with distilled water to pH 7 and dried it with anhydrous Na_2SO_4 overnight to obtain the total fatty acid methyl esters.

GC/MS Analysis of the Essential Oil. The oil was analyzed with GC/MS using a 5988A GC-MS apparatus. An SE-54 fused silica column (50 m × 0.32 mm i.d., 0.25 μm film thickness) was used with helium as carrier gas (2.8 mL/min). The GC oven temperature was kept at 40°C for 3 min, then increased to 270°C at the rate of 2.3°/min, and finally kept constant at 270° for 3 min. MS was performed at 70 eV. Interface temperature and gasification orifice temperature was 270°C with emission current at 300 mA.

In order to completely isolate the chromatogram and obtain a higher reliability of the ion-peak mass spectrum chart, we repeated the procedure three times. The conditions were the following: 1ZSZ, column temperature set at 40°C, then increased to 270°C at a rate of 3°/min, injection volume 0.2 μL; 2ZSZ, column temperature set at 40°C, then increased to 270°C at a rate of 10°/min, injection volume 0.15 μL; 3ZSZ, column temperature set at 120°C (kept for 50 min), then increased to 270°C at a rate of 10°/min, injection volume 0.1 μL. Finally, ten clear mass spectra of fatty acid methyl esters were obtained. According to the mass spectrum fragment regulation [12], we compared our results with the standard and then identified the name and content of each compound.

Studying the Function in Lowering Blood Lipid. Eighty-eight Kunming genus male mice (18–22 g weight) were provided by the Experimental Animals Centre of Lanzhou University. Ten of the mice were used for the acute toxicity experiment. These 10 mice were fed with the seed oil (5 mL/kg) and observed for two weeks to ensure that the seed oil was not harmful and could be taken safely. The remaining 78 mice were divided randomly into six groups, with 13 mice in each group. Six treatments were designed, and the groups were divided into the blank control group, the hyperlipidemia group, the group treated with lovastatin, and the seed oil treated groups at low, medium, and high dose.

Before the lovastatin and ASSO treatment the hyperlipidemic mice model must be successfully achieved. So firstly, except for the blank group, the other five groups were given a hypoglycemic diet (i.e., high-fat diet) as much as needed. The hyperlipidemic diet contained 2% cholesterol and 0.4% bile salt in the basic feeding. The blank group received a normal diet. In order to make sure that the hyperlipidemic mice model was achieved successfully, ten days later we selected three mice in each group to weigh and collected blood samples from the retrobulbar venous plexus immediately using capillary tubes with ether anesthesia and with 0.1 M EDTA as anticoagulant.

After the hyperlipidemic mice model was achieved successfully, the treatments below were conducted. The dose of lovastatin and ASSO per day for each mouse in the lovastatin and ASSO groups was determined based on weight. For the lovation group, the dose was 0.05 mg/kg/d; for the ASSO treatments, the doses were 0.10, 0.15, and 0.20 mL/10g/d for the low, medium, and high dose groups, respectively. Thirty days later, we weighed the mice and collected blood samples from the retrobulbar venous plexus immediately using capillary tubes with ether anesthesia and with 0.1 M EDTA as anticoagulant. The blood samples were allowed to clot for 30 min, and the serum was then separated by centrifugation. Plasma TG and TC levels were determined by specific kits (Biosino Bio-technology and Science, Inc.). T-SOD activity in serum was determined by the xanthine oxidase method.

All results were expressed as means ± SD for each group ($n = 10$). Data were analyzed statistically by one-way analysis of variance (ANOVA). Significant differences were at $P < 0.05$ or 0.01.

Analysis of Amino Acid. Seeds were dried at 65°C to constant mass. Dried seeds were ground to fine powder and passed through a 0.25 mm sieve. We weighed 30 mg of ground sample and put it into a tube, then added 10 mL 6 mol/L HCl. The tube was sealed, and the air in the tube was extracted. Next, we injected N_2 into the tube. The samples were hydrolyzed at 110°C for 22 h, then cooled, filtered into a volumetric flask, and diluted to 50 mL final volume. We took 1 mL samples and dried it in vacuum. We syringed it with deionized water and dried it. This cleaning process was repeated 2–3 times, then we added 1 mL 0.02 mol/L HCl in it. Amino acids were determined using an amino acid automatic analysis apparatus (Hitachi 835-50, Japan). We took another 30 mg ground sample. After it was hydrolyzed by alkali, the content of amino acid was determined by fluorescence spectrophotometry.

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