Chapter 26

Supercritical CO_2 Extracted Seabuckthorn Pulp Oil and Seed Oil Improve Blood Microcirculation

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SUMMARY

The effects of supercritical CO_2-extracted seabuckthorn seed oil (SBSO) and pulp oil (SBPO) on microcirculation were investigated using two independent experimental models. In the mouse auricular microcirculation model, topical application of SBSO and SBPO on mouse ear, once or twice per day, for three days increased the count of capillary net intersections by 17-41 per cent and venular calibers by 11-25 per cent on auricular area. Application of SBPO twice per day for three days increased the arteriolar calibers by 55 per cent. In the rat mesenteric microcirculation model, a ten-minute immersion of mesenteries in 1-2 per cent SBPO or SBSO in the Locke solution increased the count of mesenteric capillary net intersections by 22-41 per cent and venular calibers by 11-25 per cent. Immersion in 2 per cent SBPO resulted in an increase of 25 per cent in the mesenteric arteriolar calibers. These effects of seabuckthorn oils were found to be statistically significant by comparisons carried out between the corresponding values before and after treatment as well as between seabuckthorn oil-treated groups and the negative control groups, *i.e.* the group treated with distilled water in mice auricular microcirculation model and the group treated with 100 per cent Locke solution in mesenteric microcirculation model. The effects of seabuckthorn oils were dose responsive in both models. The results suggest that supercritical CO_2-extracted seabuckthorn seed and pulp oils have great potential in improving microcirculation.

Keywords: *Hippophae rhamnoides, Supercritical CO_2 extraction, Seed oil, Pulp oil and Microcirculation.*

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INTRODUCTION

The health effects of oils from seeds, berries, and fruit pulp/peel of seabuckthorn (*Hippophae rhamnoides*) have been extensively documented. The oils are known to be beneficial to the health of heart, cardio- and cerebro-vascular system, skin, mucous membranes (1). However, the mechanisms behind most of these beneficial effects are yet to be investigated. Microcirculation refers to the blood circulation of the smallest blood vessels (diameter <100 mm) of the body. Due to its crucial function in the exchange of substances, gases and liquids between tissues and blood, microcirculation plays a vital role in the physiology of the whole body.

The aim of the present study was to investigate the possible effects of supercritical CO_2 extracted seabuckthorn seed and pulp oils on microcirculation. The results may not only provide new scientific explanation of the known effects of seabuckthorn oil but also help to introduce new areas of application for the valuable oils.

MATERIALS AND METHODS

Animals and Ethical Permission

Healthy SD rats (both male and female, body weight 230 ± 20 g) and ICR mice (both male and female, body weight 20 ± 2 g) were provided by the Experimental Animal Center of Xi'an Jiao Tong University. The study was approved by the Ethical/Administration Committee of Experimental Animals, Xi'an Jiao Tong University and was carried out in 1999.

Chemicals and Test Materials and Instrumentation

Seabuckthorn seed oil (SBSO) was extracted from seeds and pulp oil (SBPO) from dried fruit flesh/peel by supercritical CO_2. Anisodamine hydrochloride injection was produced by Shanghai First Pharmaceutical Factory (Batch no. 990702), turpentine oil by Xi'an Chemical Reagent Factory (Batch no. 970903), and pentobarbital sodium by Guangzhou South Chemical Glass Company. JCD_2-A reading microscope (Hangzhou Opticla Instrument Factory, China); optical microscope (Chongqing Optical Instrument Factory, China); ocular micrometer (Shanghai Fine Instrument Factory, China).

Auricular Microcirculation of Mice (3)

Sixty ICR mice were randomly divided into six groups. After anesthetization with intraperitoneal injection of 0.4 per cent pentobarbital sodium solution (40 mg/kg), the mice were flattened on an optical microscope platform with auricle surface extended and fixed on the surface of the platform. The auricle surface was treated with paraffin before the capillary net intersection with surrounding blood vessels within an area of 1 mm^2 was counted and the arteriolar and venular calibers were measured. Then the mice were topically treated on the auricle for three days: negative control group with distilled water, once per day; SBSO low-dose group with 50 per cent SBSO emulsion once per day; SBSO high-dose group with 50 per cent SBSO emulsion twice per day; SBPO low-dose group with 50 per cent SBPO emulsion once per day; SBPO high-dose group with SBPO emulsion twice per day; positive control group with turpentine oil once per day.

Twenty minutes after the last treatment, the capillary net intersection within the same area was counted and the arteriolar and venular calibers were measured again. Difference before and after treatment was calculated and compared among the groups.

Mesenteric Microcirculation of Rats (4)

Sixty SD rats were randomly divided into six groups. After anaesthetization with intraperitoneal injection of 1.5 per cent pentobarbital sodium solution (40 mg/kg), the rats were fixed on their back on a table. A segment of the jejunum was pulled out from opened stomach; the mesentery was extended and fixed in a transparent perfusion sink so that mesentery covers the sink. The sink was then perfused with 10 ml Locke solution (37 °C). Capillary intersection with surrounding blood vessels within an area of 1 mm^2 was counted and arteriolar and venular calibers were measured. This was followed by addition of 0.1 ml distilled water to the sink of the negative control group, 0.1 ml of 50 per cent SBSO to the SBSO low-dose group, 0.2 ml of 50 per cent SBSO to the SBSO high-dose group, 0.1 ml of 50 per cent SBPO to the SBPO low-dose group, 0.2 ml of 50 per cent SBPO to the SBPO high-dose group, and 0.2 ml of 1 per cent anisodamine in the positive control group. Ten min later, the capillary intersection with surrounding blood vessels within the same area was counted and arteriolar and venular calibers were measured again. Difference before and after treatment was calculated and compared among the groups.

RESULTS AND DISCUSSION

As shown in Table 26.1, treatment with SBPO or SBSO once per day (low-dose) and twice per day (high-dose) for three days significantly increased the count of capillary intersection and the venular calibers of the selected area of auricles of mice compared to the negative control (distilled water). The arteriolar calibers were increased by application of SBPO twice per day for three days.

Table 26.1: Effects of Seabuckthorn Seed Oil and Pulp Oil on Auricular Microcirculation of Mice

Groups	*Difference Before and After Treatment*		
	CCI[a] (no./mm²)	*VC[b] (mm)*	*AC[c] (mm)*
	Mean ± SD	*Mean ± SD*	*Mean ± SD*
Negative Control	0.1 ± 0.3	0.008 ± 0.014	0.005 ± 0.014
SBPO High Dose	2.0 ± 0.9**	0.033 ± 0.023**	0.020 ± 0.018*
SBPO Low Dose	1.3 ± 0.7**	0.031 ± 0.010**	0.008 ± 0.007
SBSO High Dose	1.6 ± 0.7**	0.026 ± 0.015*	0.015 ± 0.010
SBSO Low Dose	0.8 ± 0.4**	0.025 ± 0.019*	0.012 ± 0.011
Turpentine Oil	2.6 ± 0.7**	0.055 ± 0.031**	0.023 ± 0.013**

[a]: Count of Capillary Intersection; [b]: Diameter of Venular Caliber; [c]: Diameter of Arteriolar Caliber.
*: $P < 0.05$; **: $P < 0.01$, compared with negative control group.

In the study of mesenteric microcirculation of rat, immersion of the mesentery in Locke Solution containing 1 per cent or 2 per cent SBSO and SBPO increased the count of capillary intersection and the venular calibers. The arteriolar calibers were increased only by immersion in solutions containing 0.2 per cent of SBPO (Table 26.2).

Disorder in microcirculation has been shown to be a key issue in a wide range of health problems including inflammation, cardiovascular disease and diabetes. Impeded microcirculation is also an important factor in skin problems such as couperosa and cellulite. Oxidation stress is often the major cause of damage to the microcirculation system. Antioxidants such as vitamin C and vitamin E have been shown to improve microcirculation (5).

Table 21.2: Effects of Seabuckthorn Seed Oil and Pulp Oil on Mesenteric Microcirculation of Rats

Groups	Difference Before and After Treatment		
	CCI[a] (no./mm²)	VC[b] (mm)	AC[c] (mm)
	Mean ± SD	Mean ± SD	Mean ± SD
Negative Control	0.2 ± 0.4	0.007 ± 0.012	0.009 ± 0.015
SBPO High Dose	2.0 ± 0.7**	0.099 ± 0.046**	0.039 ± 0.029*
SBPO Low Dose	1.4 ± 0.7**	0.042 ± 0.025**	0.019 ± 0.014
SBSO High Dose	1.7 ± 0.5**	0.075 ± 0.067*	0.022 ± 0.025
SBSO Low Dose	1.1 ± 0.6**	0.072 ± 0.065*	0.018 ± 0.011
Turpentine Oil	2.9 ± 0.6**	0.273 ± 0.121**	0.154 ± 0.090**

[a]: Count of Capillary Intersection; [b]: Diameter of Venular Caliber; [c]: Diameter of Arteriolar Caliber.
*: $P < 0.05$; **: $P < 0.01$, compared with negative control group.

Seabuckthorn seed oil and pulp oil are rich in natural tocopherols, tocotrienols, and carotenoids, which synergistically protect the cellular components from oxidation damage (2, 6). In the present study, seabuckthorn pulp oil showed better effect in increasing the diameter arteriolar caliber. This might have been due to a stronger antioxidant activity resulting from the higher level of carotenoids in the pulp oil compared to the seed oil.

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