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Fitoterapia 73 (2002) 644–650

FITOTERAPIA

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Effects of sea buckthorn (*Hippophaë rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats

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Received 17 May 2002; accepted in revised form 13 September 2002

Abstract

Oils from sea buckthorn (*Hippophaë rhamnoides* L.) seeds and berries have traditionally been used in the treatment of disorders of skin and mucosa in China. Compared with the negative control, oral administration of CO_2-extracted seed and pulp oils, 7.0 ml · kg^{-1} · day^{-1} significantly reduced ulcer formation in water-immersion ($P<0.05$) and reserpine-induced ($P<0.01$) models in rats. In addition, administration of the two oils, 3.5 ml · kg^{-1} · day^{-1}, significantly reduced the index of pylorus ligation-induced gastric ulcer ($P<0.05$) and sped up the healing process of acetic acid-induced gastric ulcer ($P<0.01$). The results suggested that the CO_2-extracted sea buckthorn seed and pulp oils have both preventive and curative effects against experimental gastric ulcers in rats.

Keywords: *Hippophaë rhamnoides*; Sea buckthron seed oil; Sea buckthorn pulp oil; Experimental models of gastric ulcer

1. Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) is a Euro–Asian wild, newly cultivated, edible berry with exceptionally high contents of nutrients and phytochemicals

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0367-326X/02/$ - see front matter
PII: S0367-326X(02)00221-6

Table 1
Major fatty acids (weight percentage), sitosterol, carotenoids and tocopherols + tocotrienols in *H. rhamnoides* seed and pulp oils

	Fatty acids (%)							Sitosterol	Carotenoids	Tocopherols + tocotrienols
Oils	16:0	16:1 (n-7)	18:0	18:1 (n-9)	18:1 (n-7)	18:2 (n-6)	18:3 (n-3)	g/kg oil	g/kg oil	g/kg oil
Seed	11.3	4.4	2.6	18.9	3.2	34.1	24.9	5.6	0.3	2.1
Pulp	33.4	24.9	1.0	26.2	7.3	5.1	1.6	14.0	1.2	2.6

such as lipids, water and fat soluble vitamins, and flavonoids [1–3]. The total antioxidative capacity of the berry is due to the presence of vitamins C and E, carotenoids as well as enzymes such as various superoxide dismutase isoenzymes [4–6]. The berries have a long history of application (more than 1000 years) in Tibetan and Mongolian medicines in the treatment of various diseases. A wide spectrum of positive physiological effects of the berries and berry products have been suggested by animal experiments and clinical investigations [7,8]. Oils extracted from sea buckthorn berries have been used for treating scalds, burns and other skin injuries in both man and animals [7–12]. Research has been carried out to test the effects of sea buckthorn oils on gastric ulcer [13–18]. However, contradictory results have been reported [13–16].

In most previous investigations, the oils were extracted with organic solvents. Recently, supercritical CO_2 extraction of natural oils has been increasing due to the absence of solvent residue in the extracted oils. In the present study, the effects of supercritical CO_2-extracted sea buckthorn seed and pulp oils on gastric ulcer were investigated for the first time.

2. Experimental

2.1. Plant material

Wild sea buckthorn (*H. rhamnoides* subsp. *rhamnoides*) berries were collected in Romania in August 1997.

2.2. Preparation of oil

Sea buckthorn oils were extracted by supercritical CO_2 from seeds (seed oil, SO) and soft part (pulp oil, PO). The extractions were carried out at a CO_2 density of ca. 0.9 g/ml. The oils were stored in CO_2 at 3 °C until used. The fatty acid composition and the contents of sitosterol, carotenoids, tocopherols and tocotrienols of the oils are shown in Table 1.

2.3. Drugs

Cimetidine (batch no. 97030508) was produced by Tianjin Smith Kline & French Laboratories Ltd. (Tianjin, PR China), and reserpine (batch no. 960802) by The

Red Flag Pharmaceutical Factory of Shanghai Medical University (Shanghai, PR China).

2.4. Animals

Sprague–Dawley rats, both male and female weighing 170–230 g, were provided by The Experimental Animal Center of Xi'an Medical University (Xi'an, PR China). They were housed in standard environmental conditions and fed with rodent diet with water ad libitum. The Administration Committee of Experimental Animals, Xi'an Medical University approved the experiment.

2.5. Antiulcer activity

Rats fasted for 24 h were randomly divided into six groups. Control solutions and different doses of sea buckthorn seed oil and pulp oil were orally administered to these groups as follows: (1) negative control group, 7 ml/kg·day^{-1} 4% Tween 80 water solution; (2) positive control group, cimetidine, 80 mg/kg·day^{-1} dissolved in 7 ml of 4% Tween 80 water solution; (3) SO group, oil 7 ml/kg·day^{-1} and (4) SO group, oil 3.5 ml/kg·day^{-1}, administered as 50% oil emulsions in 4% Tween 80 water solution; (5) PO group, oil 7 ml/kg·day^{-1} and (6) PO group, oil 3.5 ml/kg·day^{-1}, administered as 50% oil emulsions in 4% Tween 80 water solution.

All animals were treated, once a day, for 7 days.

2.5.1. Ulcer induction with water immersion stress

Animals were treated as described in Section 2.5. One hour after the last treatment, the rats were fixed on the immobolizing board and immersed into water (23 °C) up to the xiphoid level for 20 h. At the end of the immersion period, the rats were killed by dislocation of the cervical vertebrae. The stomachs were removed, slightly inflated by injecting 8 ml of 1% formalin solution by esophageal injection (pylorus and cardia ligated) and kept in 1% formalin solution for 30 min. Subsequently, the stomach was incised along the greater curvature and the ulcer examined under a magnifying glass (5×) [19].

2.5.2. Ulcer induction with reserpine

Animals were treated as described in Section 2.5. Thirty minutes after the last administration, reserpine was injected intraperitoneally. Eighteen hours later, the rats were killed, the stomachs were removed, incised along the greater curvature [20] and the ulcer index calculated [19].

2.5.3. Ulcer induction by pylorus ligation

Animals were treated as described in Section 2.5. Three hours after the last administration, the animals were anesthetized with diethyl ether, and the pylorus ligated. After 18 h, the rats were killed, the stomachs removed, opened, treated with formalin and the ulcer examined under magnifying glass (5×) [21].

Table 2
Effect of *H. rhamnoides* seed and pulp oils on the water-immersion stress-induced gastric ulcer in rats

Group	Dose (ml/kg) day^{-1}	Ulcer index mean ± S.D.	Inhibition%
Control		5.50 ± 1.78	
Seed oil	7.0	3.36 ± 1.50**	38.9
	3.5	4.30 ± 1.42	21.8
Pulp oil	7.0	3.72 ± 1.42*	32.4
	3.5	4.33 ± 1.56	21.3
Cimetidine	80.0 mg	3.25 ± 1.84	40.9

$n = 10$.
* $P < 0.05$ compared with control group.
** $P < 0.01$ compared with control group.

2.5.4. Ulcer induction with acetic acid

Animals were anaesthetized with diethyl ether, the stomach was opened and 0.2 ml of acetic acid was directed to the serousal surface via a glass tube (5-mm diameter, 20-mm long) tightly placed on it.

After 1.5 min, acetic acid was removed with cotton sticks, and the stomach closed [21].

Thereafter, the rats were given a normal water and food supply. On the second day, the rats were grouped, and administered oils or control solutions as described in Section 2.5. The oil administration lasted for 12 days. On the 13th day, the rats were killed, the stomachs removed, treated and the area (mm^2) of the ulcers was measured and the ulcer indices evaluated [22].

2.6. Statistical analyses

The statistical analyses were carried out with the statistical program SPSS 7.5. The differences between the ulcer index in different groups were compared with Independent Samples *t*-test.

3. Results

The seed oil, pulp oil and cimetidine showed protective effects against different models of ulcer formation. As reported in Tables 2 and 3, it was recognized that both oils showed a dose–response inhibition on ulcers induced by water-immersion and reserpine with an inhibition ranging from 21 to 39% and 11 to 70%, respectively. On the contrary, only the SB pulp oil showed a dose-dependent inhibition (22–44%) on pylorus-ligation-induced ulcer. The SB seed oil showed an inhibition of approximately 20% for both the tested doses (Table 4).

Also, on the acetic acid-induced ulcer model, the curative effect of sea buckthorn oils resulted independently from the dose used. In fact, the inhibition was found to be approximately 45% for all the tested dose compared to the 51% showed by cimetidine (Table 5).

Table 3
Effect of *H. rhamnoides* seed and pulp oils on reserpine-induced gastric ulcer in rats

Group	Dose (ml/kg) day^{-1}	Ulcer index mean±S.D.	Inhibition%
Control		4.33±1.92	
Seed oil	7.0	1.32±0.98**	69.5
	3.5	3.83±2.22	11.5
Pulp oil	7.0	2.09±1.74**	51.7
	3.5	3.41±1.56	21.2
Cimetidine	80.0 mg	2.40±1.90*	44.6

$n=10$.
* $P<0.05$ compared with control group.
** $P<0.01$ compared with control group.

Table 4
Effect of *H. rhamnoides* seed and pulp oils on pylorus-ligation-induced gastric ulcer in rats

Group	Dose (ml/kg) day^{-1}	Ulcer index mean±S.D.	Inhibition%
Control		4.92±0.29	
Seed oil	7.0	3.92±1.44*	20.3
	3.5	3.83±1.80*	22.2
Pulp oil	7.0	2.75±1.54**	44.1
	3.5	3.83±1.19*	22.2
Cimetidine	80.0 mg	3.42±2.11*	30.5

$n=10$.
* $P<0.05$ compared with control group.
** $P<0.01$ compared with control group.

Table 5
Effect of *H. rhamnoides* seed and pulp oils on pylorus-ligation-induced gastric ulcer in rats

Group	Dose (ml/kg) day^{-1}	Ulcer index mean±S.D.	Inhibition%
Control		6.45±1.90	
Seed oil	7.0	3.54±2.50*	45.1
	3.5	3.61±1.89*	44.0
Pulp oil	7.0	3.18±1.71*	50.7
	3.5	3.60±2.40*	44.2
Cimetidine	80.0 mg	3.11±3.04*	51.8

$n=10$.
* $P<0.01$ compared with control group.

4. Discussion

Che et al. reported protective and curative effects of sea buckthorn seed oil (2 ml/kg, once a day, or 5 ml/kg, once a day, for 7 days) for water immersion,

reserpine and acetic acid-induced gastric ulcers in rats [15]. On the contrary, Jiang et al. did not show a protective effect of the seed oil on water-immersion or acute reserpine models, while the pulp oil did not show a significant curative effect on acetic acid-induced gastric ulcer in rats. The varying results obtained in the two investigations may have been due to the difference in the oil extraction methods which were not defined by the authors [16].

The results of the present study showed both protective and curative effects of the CO_2-extracted sea buckthorn seed and pulp oils on the gastric ulcers in four experimental models in rats. The composition of the CO_2 extracted oils in our experiments was probably not the same as those of the oils applied in the earlier investigations. Even though the fatty acid compositions of the two oils are very divergent (Table 1), no significant differences were observed in the gastric protective effects of the two oils. The results suggest that the antiulcer effects of the tested sea buckthorn oils may not have been due to their fatty acids only. The oils contained high levels of β-carotene, α-tocopherol and β-sitosterol (Table 1). β-Sitosterol and β-sitosterol-β-D-glucoside have been shown to have curative effects on acetic acid-induced gastric ulcers. A protective effect of β-sitosterol glucoside against water-immersion-induced ulcers in rats has also been shown [23,24]. β-Sitosterol is also reported to increase the gastric protective activity of unsaturated phospholipids [25]. In addition, evidence exists showing that administration of sea buckthorn oil inhibits lipid peroxidation in gastric mucosa in experimental models of gastric ulcer in rats [26]. The antiulcer activity of sea buckthorn oils may be related to an increase in the hydrophobicity of the mucosal surface, retarding the gastric emptying process, inhibiting proteolytic activity in gastric liquid and promoting the wound reparation processes of mucosa [7,8,17,18,23].

Acknowledgments

Aromtech Oy (Tornio, Finland) is acknowledged for providing the oil capsules and sharing the expenses of the experiment.

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