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Full Length Research Paper

Effects of sea buckthorn (*Hippophaë rhamnoides* L.) pulp oils on the gastric secretion, gastric emptying and its analgesic activity

Jianfeng Xing[1], Sun Jinyao[2], Sasa Hu[2], Bingwen Wang[1], Yalin Dong[2]*, Baoru Yang[3] and Heikki P. Kallio[3]

[1]Department of Pharmacy, College of Medicine, Xi′an Jiaotong University, Xi′an, 710061, China.
[2]Department of Pharmacy, the First Affiliated Hospital of Medicine College, Xi′an Jiaotong University, Xi′an, 710061, China.
[3]Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland.

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Sea buckthorn (*Hippophaë rhamnoides* L.) pulp oils (SBPO) were evaluated for its effect on gastric secretory function, gastric emptying and analgesic activity, as a potential treatment for stomach discomfort and gastric ulcers. The actions of SBPO on gastric acid, pepsin and mucus secretions were studied in Shay rats. SBPO, when administered for 7 days at the dose of 3.5 and 7.0 ml/kg, caused a significant decrease in gastric volume, total acidity and pepsin output. There was also a significant increase in gastric mucus production. Gastric emptying was studied using a carboxymethyl cellulose solution as a non-nutrient meal in mice. SBPO caused a significant decrease in gastric emptying. The antinociceptive effect was evaluated using the acetic acid induced writhing test. SBPO significantly inhibited the number of writhing responses. These results suggest effectiveness of SBPO in stomach discomfort and gastric ulcers.

Key words: *Hippophaë rhamnoides*, Sea buckthorn pulp oil, gastric secretion, gastric emptying, antinociceptive effect.

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.), a member of the *Elaeagnaceae* family, is naturally distributed over Asia and Europe (Rousi, 1971). Different parts of the Sea buckthorn, especially its berries, have been used in traditional medicine in various countries (Guliyev et al., 2004). Its fruit contain carotenes (a, b and d), Vitamins C and E, riboflavin, folic acid, tannins, sugar, glycerides of palmitic, stearic and oleic acids, polyphenols and some essential amino acids (Beveridge et al., 1999). These compounds possess biological and therapeutic activity, including antioxidant, immunomodulatory, anti-ulcerogenic, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue repair (Geetha et al., 2003; Xing et al., 2002; Gao et al., 2003; Basu et al., 2007; Chawla et al., 2007; Upadhyay et al., 2009). Oils from berries and seeds are used in the treatment of gastrointestinal malfunction, liver diseases, inflammatory diseases, erosion of the cervix uteri, burns and frostbite (Suryakumar and Gupta, 2011). The curative and preventive effects of Sea buckthorn against gastric ulcers in rats have been reported (Suleyman et al., 2001). In previous investigations, these oils were extracted using organic solvents. Recently, the use of supercritical CO_2 extraction has increased because solvent residue is absent in the extracted oils. Previous researches demonstrated that CO_2-extracted sea buckthorn seed and pulp oils had anti-ulcerogenic effects. Results show that these oils reduce ulcer formation in water-immersion stress-, reserpine-, and pylorus ligation-induced gastric ulcer models. They also accelerate healing of acetic acid-induced gastric ulcers (Xing et al., 2002).

The pathophysiology of gastric ulcers generally focuses on the imbalance between aggressive and protective

*Corresponding author. E-mail: dongyalin@medmail.com.cn. Tel: +86-29-85323241

factors in the stomach, such as acid and pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors (Lima et al., 2006). Gastric ulcer treatment consists of eliminating pain, existing lesions and the prevention of new lesion formation. Current therapeutic agents are anti-acids, anti-secretory agents, agents protecting the mucus, cytoprotective agents and substances that delay gastric emptying (Meyer et al., 2002). It has been reported that sea buckthorn oils increased gastric acidity and peptic activity (Huang et al., 2002). In contrast, it also has been reported that sea buckthorn oils reduced peptic activity; meanwhile, there was no significant change in gastric acidity (Zhou et al., 1994). In addition, the effect of sea buckthorn oil on the gastric mucus secretion has been not reported. In the present study, the effects of supercritical CO_2-extracted sea buckthorn pulp oil on gastric secretion, gastric emptying and analgesic activity were investigated for the first time.

MATERIALS AND METHODS

Preparation of oil

Sea buckthorn pulp oils (SBPO), extracted by supercritical CO_2 from the soft parts of sea buckthorn berries, was provided by the Department of Biochemistry and Food Chemistry of Turku University (Turku, Finland). 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 in the oil were measured as described previously (Ranjith et al., 2006) and (Table 1).

Drugs and chemicals

Cimetidine was obtained from GlaxoSmithKline (China) Pharmaceutical Co., Ltd. Atropine and aspirine were obtained from Xi'an Lijun Pharmaceutical Co., Ltd. Bovine haemoglobin and alcian blue 8 GX were obtained from Sigma. All other chemicals were made in China and of analytical grade.

Animals

Sprague-Dawley rats (170 to 190 g) and Kunming mice (18 to 24 g) of both sexes were obtained from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). The animals were housed under a 12-h light–dark cycle at a constant ambient temperature of 22 to 25°C and a relative humidity of 40 to 60%, with normal rat chow and water *ad libitum*. They were allowed to acclimatize for one week before the experiments were started. The university's ethics review committee approved the animal experimental protocol.

Gastric secretion in Shay rats

Gastric juice was collected using the pylorus-ligated rat model, first described by Shay et al. (1954). Rats were randomly divided into four groups, each comprising nine rats, and treated orally with distilled water, cimetidine (80 mg/kg) and SBPO (3.5 and 7.0 ml/kg) for seven days. Animals were fasted over night, and the drugs were administered orally 1 h before starting the experiment. The pylorus was tied under diethyl ether anesthesia. Care was taken not to damage the blood supply. Four hours later, the animals were killed using an overdose of anesthetic. The abdomen was opened and the cardia was ligated. The stomachs were removed and the gastric content collected and drained into a graduated centrifuge tube and centrifuged at 2000 g for 10 min. The supernatant was collected and used for the estimation of volume of gastric juice, total acid output, peptic output, and gastric wall mucus.

Total acid output was determined by titrating with 0.01 M sodium hydroxide, using phenolphthalein as indicator and was expressed as mEq/h. Peptic activity was determined using bovine hemoglobin as substrate and was expressed as µmol of tyrosine/h as output (Bhattacharya et al., 2006). Gastric wall mucus was quantitatively measured as described by Corne et al. (1974). The stomachs were removed and were soaked in 0.1% Alcian blue 8 GX (AB) solution for 2 h. The uncomplexed dye was removed by two successive washes at 15 and 45 min in 0.25 M aqueous sucrose solution. Dye complexes with gastric wall mucous were extracted by immersion in 10 ml of 0.5 M $MgCl_2$ for 2 h. The resulting blue solution was mixed with equal volumes of diethyl ether and the absorbency of the aqueous phase was measured by spectrophotometry at 615 mm. The amount of gastric mucus was expressed as mg Alcian blue (AB)/g wet tissue.

Gastric emptying in mice

Gastric emptying was measured according to the method described by Du et al. (2007) with slight modification. Mice were deprived of food for 12 h in wire-bottom cages individually to prevent coprophagy and with free access to water. The test meal consisted of a non-nutrient meal of 1.5% sodium carboxymethyl cellulose dissolved in 0.1% methyl orange, a non-absorbable and easily detectable marker. Forty minutes after oral treatment of mice with distilled water and SBPO (15 and 30 ml/kg), or atropine (2 mg/kg) of intraperitoneal injection, the animals received orally 0.2 ml of the test meal and were sacrificed 20 min later. Under a laparatomy, the stomach was excised after ligation of the pylorus and the cardia. The stomach was cut into several pieces in 10 ml distilled water to collect the gastric contents, including methyl orange. The gastric contents were adjusted to pH 6.0 to 6.5 with 5% $NaHCO_3$ solution, and then centrifuged at 2000 g for 10 min, and the absorbance of supernatant was measured at 420 nm. Gastric emptying was calculated according to the following formula:

Gastric emptying (%) = (1–B/A) × 100 (A: absorbance of methyl orange recovered from the stomach immediately after administration of the test meal containing methyl orange; B: absorbance of methyl orange remaining in the stomach 20 min after administration of the test meal containing methyl orange).

Acetic acid-induced writhing test

This test was carried out by using the method described by Sawadogo et al. (2006) with minor modification. Forty mice were randomly divided into four groups, ten mice each group. Writhing was induced by intraperitoneal injection of 0.9% acetic acid. The mice were orally administered distilled water, aspirin (200 mg/kg) and SBPO (15 and 30 ml/kg), respectively, before forty minutes of intraperitoneal injection of acetic acid (10 ml/kg). The number of writhing reflexes was counted during the following 15 min

Statistical analyses

All statistical analyses were performed with SPSS version 11.5 for

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

Fatty acids (%)							Sitosterol	Carotenoids	Tocopherols+tocotrienols
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
33.4	24.9	1.0	26.2	7.3	5.1	1.6	14.0	1.2	2.6

Windows. The parameters were compared by one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. All results were expressed as mean ± SD. Statistical significance was set at $P < 0.05$.

RESULTS

Effects of SBPO on the gastric secretion and gastric wall mucus in 4 hour pylorus-ligated rats

In control rats, pylorus ligation for 4 h resulted in accumulation of 7.6 ± 1.8 ml of gastric secretion and a total acid output of 81.0 ± 15.5 mEq/h and a pepsin output of 4.7 ± 1.0 µmol tyrosine/h (Figure 1). The volume of gastric secretion in the rats treated with 3.5 and 7 ml/kg of SBPO significantly reduced to 5.5 ± 1.0 ml and 5.6 ± 1.9 ml, respectively. A significant decrease in total acid output and pepsin output were observed in the rats treated with SBPO (3.5 and 7.0 ml/kg). A similar observation was done with gastric wall mucus. The treatment of rats with SBPO significantly increased the alcian blue binding capacity of gastric wall mucus as compared to control rats. These findings are summarized in Figure 1.

Effect of SBPO on gastric emptying in mice

As shown in Figure 2, SBPO delayed gastric emptying compared with control group. Twenty minutes after ingestion of the test meal in control mice, gastric emptying rate in control mice was 83.7%. Treatment with SBPO (15 and 30 ml/kg) significantly reduced gastric emptying rate to 43.4 and 42.9%, respectively.

Effect of SBPO on writhing reflex of mice

In the acetic acid-induced writhing test, intraperitoneal injection of acetic acid evidently resulted in writhing reflexes of mice. SBPO significantly reduced the number of writhing responses in a dose-dependent manner within 15 min of injection of acetic acid. The writhing number of the mice given high dose of SBPO (30 ml/kg) was lower than that of the mice received aspirin (Figure 3).

DISCUSSION

Gastric ulcer is the most common gastrointestinal disorder in clinical practice today. Although the etiology of gastric ulcer is still debated, it is accepted that ulcers are due to an imbalance between aggressive and protective factors in the stomach, such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors (Jaiswal et al., 2011). Consequent reduction of gastric acid production, as well as reinforcement of gastric mucosal protection, has been the major therapeutic approaches of gastric ulcer disease (Lakshmi et al., 2010).

To regain balance between aggressive and protective factors, different therapeutic agents including plant extracts are used. The gastric protective effect of SBPO extracted by supercritical CO_2 from the soft parts of sea buckthorn berries in different ulcerogenic models in water-immersion stress-, reserpine-, pylorusligature- and acetic acid-induced gastric ulcers in rats has been investigated in previous studies. These studies have demonstrated that SBPO has both preventive and curative effects against experimental gastric ulcers in rats (Xing et al., 2002). However, the possible antiulcerogenic mechanisms of SBPO involved in this action have not yet reported. In the present study, the effects of SBPO on gastric secretion, gastric motility and analgesic activity were investigated for the first time.

In the present study, pre-treatment with SBPO reduced the gastric volume, total acidity and pepsin output in 4 h pylorus ligated rats. Increased gastric acidity and pepsin output are considered to be important factors in pathogenesis of gastric ulcers and are often termed the ‘aggressive factor’ (Goa and Monk, 1987). Drugs with the ability to reduce gastric acid and pepsin secretion have been shown to attenuate ulcerogen induced gastric mucosal damage (Takeuchi et al., 2003). Furthermore, our results revealed that SBPO significantly elevated the gastric wall mucus. The mucus gel adhering to the gastric mucosal surface

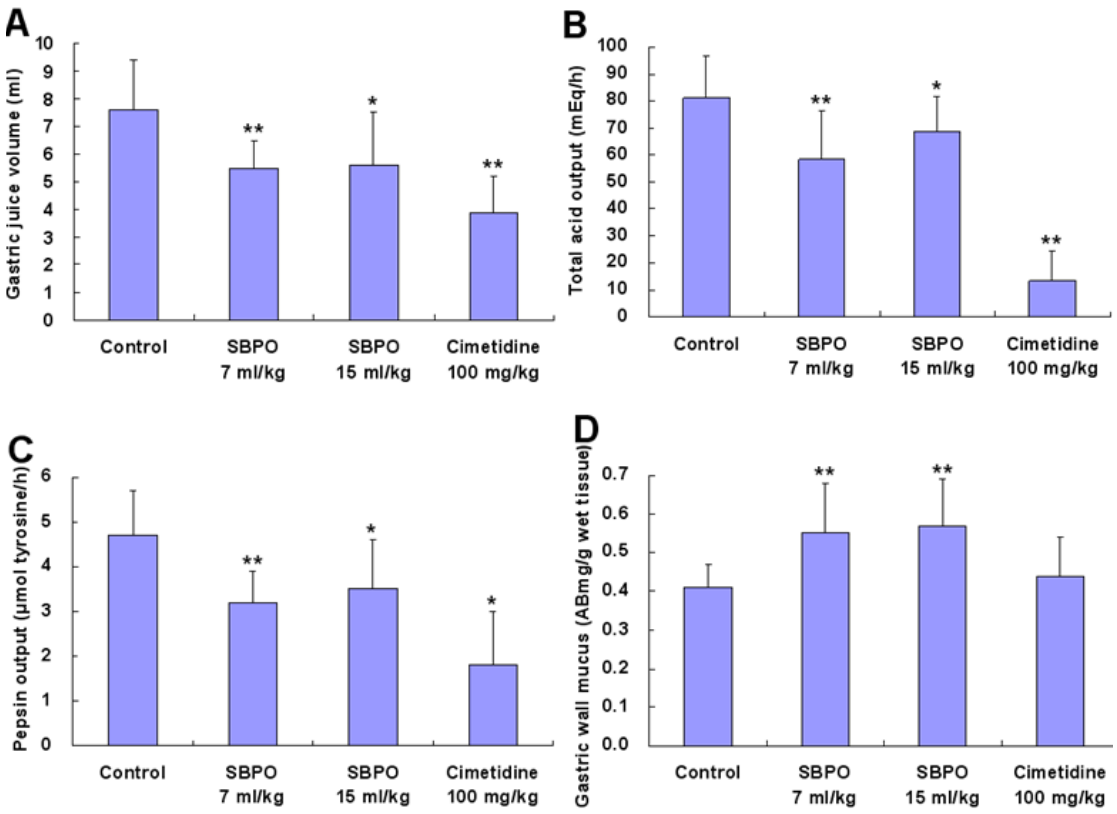

Figure 1. Effects of SBPO on the gastric secretion and gastric wall mucusin in pylorus-ligated rats. The volume of gastric secretion in the rats treated with SBPO significantly reduced (A); A significant decrease in total acid output (B) and pepsin output (C) were observed in the rats treated with SBPO; The alcian blue binding capacity of gastric wall mucus in the rats treated with SBPO significantly.

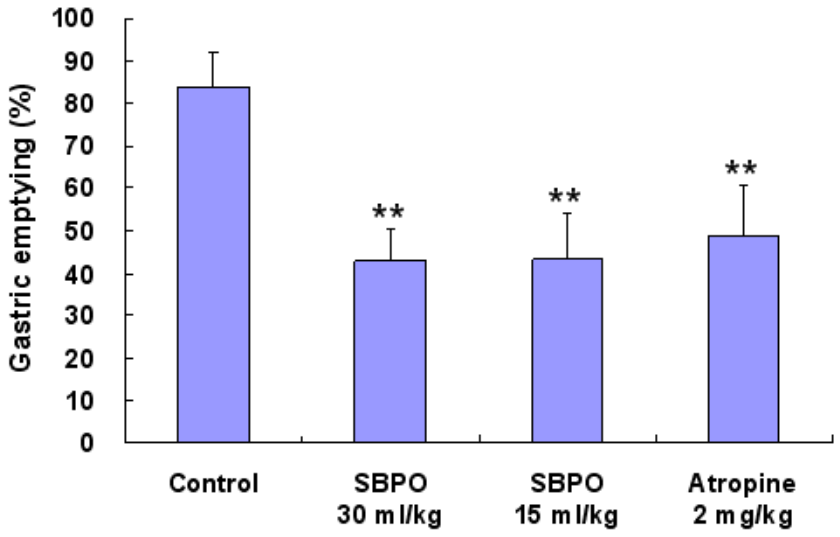

Figure 2. Effect of SBPO on gastric emptying in non-nutrient meal-loaded mice. Values are the mean ± SD for ten mice. ** P < 0.01 compared with control group.

protects the underlying epithelium against acid, pepsin (Bell et al., 1985) and necrotizing agents such as ethanol and indomethacin (Allen et al., 1987). The gastric mucus coat is thought to be important in facilitating the repair of the damaged gastric epithelium (Wallace and Whittle, 1986). The present study showed that SBPO significantly

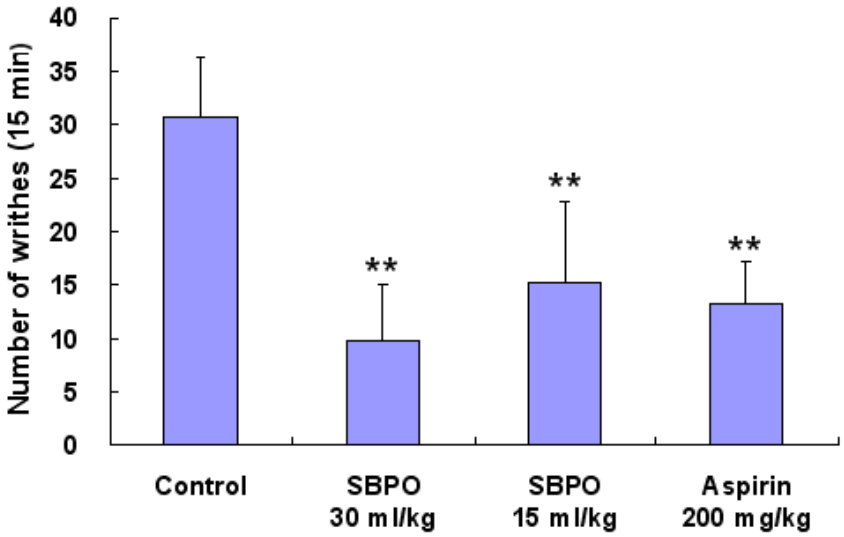

Figure 3. Effect of SBPO on writhing induced by acetic acid in mice. Values are the mean ± SD for ten mice. ** P < 0.01 compared with control group.

significantly delayed gastric emptying. It has been reported that inhibiting gastric motility may offer mucosal protection (Takeuchi et al., 1988). In contrast, it also has been reported that a delay in gastric emptying is an important factor gastric ulcer development (Gupta et al., 1989). Although there is considerable controversy about the role of gastric motility in the prevention of gastric mucosal injury (Mersereau and Hinchey, 1981; Takeuchi et al., 1987; Takeuchi et al., 1988; Takeuchi et al., 1989), the inhibited gastric motility may induce the flattening of mucosal folds, decreasing susceptibility of mucosal folds to corrosive action of irritants, thereby offering mucosal protection.

The results of the present study showed that SBPO decreases output of gastric acid and pepsin increases the gastric wall mucus. The observed effects of SBPO may have been due to its fatty acids. SBPO is rich in fatty acids, which are potent stimuli for the release of enterohormones and modification of gastric motility (McLaughlin et al., 1999). The enterohormones, such as cholecystokinin, secretin, somatostatin, pancreatic polypeptide, were able to inhibit gastric secretion. It may be concluded that gastro-protection offered by SBPO is mediated through its effect on mucus production, its antiacid secretory properties, and inhibition of gastric motility. The results of the present study suggest the possibility of using SBPO in the treatment of stomach discomfort and gastric ulcers.

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