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Pancreatic lipase inhibitory activity of *taraxacum officinale in vitro* and *in vivo*[*]

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Abstract

Obesity has become a worldwide health problem. Orlistat, an inhibitor of pancreatic lipase, is currently approved as an anti-obesity drug. However, gastrointestinal side effects caused by Orlistat may limit its use. In this study the inhibitory activities of dandelion (*Taraxacum officinale*) against pancreatic lipase *in vitro* and *in vivo* were measured to determine its possible use as a natural anti-obesity agent. The inhibitory activities of the 95% ethanol extract of *T. officinale* and Orlistat were measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate at concentrations of 250, 125, 100, 25, 12.5 and 4 μg/ml. To determine pancreatic lipase inhibitory activity *in vivo*, mice (n=16) were orally administered with corn oil emulsion (5 ml/kg) alone or with the 95% ethanol extract of *T. officinale* (400 mg/kg) following an overnight fast. Plasma triglyceride levels were measured at 0, 90, 180, and 240 min after treatment and incremental areas under the response curves (AUC) were calculated. The 95% ethanol extract of *T. officinale* and Orlistat, inhibited, porcine pancreatic lipase activity by 86.3% and 95.7% at a concentration of 250 μg/ml, respectively. *T. officinale* extract showed dose-dependent inhibition with the IC_{50} of 78.2 μg/ml. A single oral dose of the extract significantly inhibited increases in plasma triglyceride levels at 90 and 180 min and reduced AUC of plasma triglyceride response curve ($p<0.05$). The results indicate that *T. officinale* exhibits inhibitory activities against pancreatic lipase *in vitro* and *in vivo*. Further studies to elucidate anti-obesity effects of chronic consumption of *T. officinale* and to identify the active components responsible for inhibitory activity against pancreatic lipase are necessary.

Key Words: mouse, pancreatic lipase, *Taraxacum officinale*, triglyceride

Introduction

Obesity, defined as the excessive accumulation of body fat that may impair health, has become a worldwide epidemic (Mokdad *et al.*, 2003). Obesity is a major risk factor for cardiovascular disease including heart disease and stroke, diabetes mellitus, musculoskeletal disorders such as osteoarthritis, and some cancers such as breast, endometrial, prostate, and colon cancer (Aronne, 1998). The fundamental cause of obesity is an energy imbalance between calorie intake and expenditure. Therefore, inhibition of digestion and absorption of dietary fat, the most concentrated source of energy, can be useful in treatment of obesity. In fact, Orlistat, a potent competitive inhibitor of gastric and pancreatic lipase, is available as an anti-obesity drug (Ballinger & Peikin, 2002; Hochuli *et al.*, 1987; Jandacek & Woods, 2004; Weibel *et al.*, 1987). However, this medication is associated with a greater incidence of gastrointestinal side effects (Birari & Bhutani, 2007; Weigle, 2003). Therefore, numerous studies have evaluated natural products with pancreatic lipase inhibitory activity, including plant materials, as an alternative anti-obesity agent (Arai *et al.*, 1999; Han *et al.*, 2001; Han *et al.*, 2002; Kim, 2007; Kurihara *et al.*, 2003; Kurihara *et al.*, 2006). Dietary teasaponin (Han *et al.*, 2001), saponins from Platycodi Radix (Han *et al.*, 2002), and onion skin extract (Kim, 2007) were reported to decrease postprandial plasma triglyceride response after lipid load and showed anti-obesity effects in animals fed high fat diets.

Dandelion (*Taraxacum officinale*) is a herbaceous perennial plant of the family Asteraceae (Compositae). *T. officinale* has been used as a phytomedicine due to its choleretic, antirhemetic, diuretic, and anti-inflammatory properties (Bisset, 1994). It has been reported that *T. officinale* exhibits anti-inflammatory activity (Mascolo *et al.*, 1987) and anti-tumor activity (Williams *et al.*, 1996). Antioxidant activity of *T. officinale* has been demonstrated *in vitro* (Hu & Kitts, 2005) and *in vivo* (Cho *et al.*, 2003). *T. officinale* has been reported to have hypolipidemic effects in rats fed a high cholesterol diet (Cho *et al.*, 2000) and streptozotocin-induced diabetic rats (Cho *et al.*, 2002).

However, the effect of this plant on the activity of pancreatic lipase has not yet been examined. Thus, in this study we measured the pancreatic lipase inhibitory activity of *T. officinale in vitro* and *in vivo* to evaluate its possible use as an anti-obesity agent.

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Materials and Methods

Reagents

Orlistat was obtained from Roche Korea (Seoul, Korea). Corn oil was purchased from Cheiljedang (Seoul, Korea). Porcine pancreatic lipase, 4-methylumbelliferyl oleate (4-MU oleate), as well as the assay kit for triglycerides and all other reagent grade chemicals were purchased from Sigma (St. Louis, MO, USA).

Preparation of the ethanol extract

Young leaves of *T. officinale* were obtained from a local market in Changnyung-gun, Korea. The leaves were freeze-dried, powdered, and extracted with ten volumes of 95% ethanol for 12 hr three times at room temperature. The solvent was removed by rotary evaporation at 40℃.

Measurement of pancreatic lipase inhibitory activity in vitro

The inhibitory activity against pancreatic lipase was measured using 4-MU oleate as a substrate (Thompson, 1978). The reaction mixture consisted of 50 μL 0.1 mM 4-MU oleate, 20 μL McIlvane buffer (0.1 M citrate-Na_2 HPO_4, pH 7.4), and 5 μL of sample solution. Porcine pancreatic lipase (25 μL) was added to the reaction mixture and the final volume was adjusted to 0.1 ml. After the mixture was incubated at 37℃ for 10 min, the amount of 4-MU released by the lipase was measured using a fluorescence multi-detection reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) at an excitation wavelength of 320 nm and an emission wavelength of 450 nm. The inhibitory activities of the *T. officinale.* extract and Orlistat, a positive control against pancreatic lipase were measured at concentrations of 250, 125, 100, 25, 12.5 and 4 μg/ml. The measurements were performed in triplicate, and the IC_{50} value, the concentration of the extract that results in 50% inhibition of maximal activity was determined.

Animals

Five week-old male ICR mice weighing between 20 and 25 g were purchased from Bio Genomics, Inc. (Seoul, South Korea). All animals were fed a commercial chow *ad libitum* for 2 wk after arrival. Mice were housed individually in stainless steel wire-bottomed cages and located in a room where temperature (23-27℃), humidity (50-60%), and lighting cycle (12 hr light/dark cycle) were controlled. Mice (28-35 g, n=16) were then randomly divided into two groups.

Measurement of plasma triglyceride levels after oral administration of a lipid emulsion to mice

Following an overnight fast, the animals were fed corn oil emulsion (5 ml/kg) alone or oil emulsion with 95% ethanol extract of *T.officinale* (400 mg/kg). The oil emulsion was composed of 80 mg cholic acid, 2 mg cholesteryl oleate, 6 ml saline, and 6 ml corn oil. Food was withheld during the test. Blood samples were collected from the ophthalmic venous plexus 0, 90, 180, and 240 min after the oral administration and centrifuged at 2,000 x *g* for 15 min. Plasma triglyceride levels were measured using a commercial triglyceride assay kit and were expressed as increments from the baseline. Incremental areas under the response curves (AUC) were calculated using the trapezoidal rule with fasting levels as the baseline. The experiments were performed according to the guidelines of animal experimentation approved by the Animal Resource Center at Inje University, Korea.

Statistics

All data are expressed as mean ± standard deviation (SD). All statistical analyses were performed using SAS (version 8.02). Differences in incremental plasma triglyceride levels and AUC between control group and *T. officinale* group were assessed using Student's t-test. Significance was defined as $p<0.05$.

Results

Dose-dependent inhibition of pancreatic lipase activity

The inhibitory activities of 95% ethanol extract of *T. officinale* against porcine pancreatic lipase are shown in Fig. 1. The *T. officinale* extract inhibited pancreatic lipase activity by 86.3,

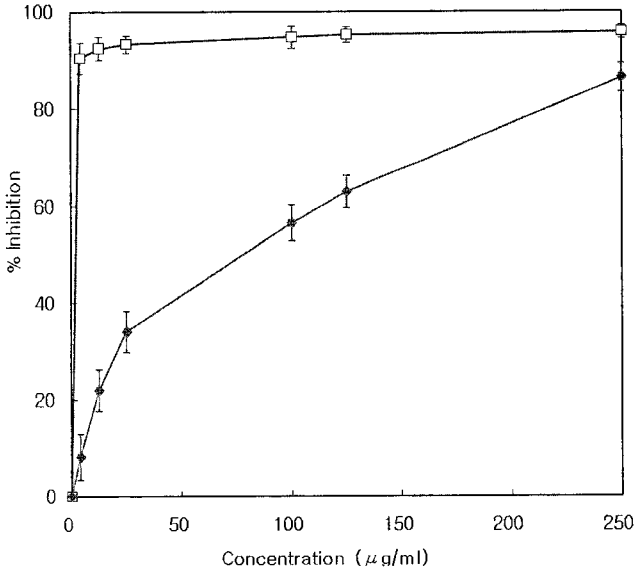

Fig. 1. Dose-dependent inhibition of pancreatic lipase activity of *Taraxacum officinale*. extract and Orlistat. The inhibitory activities of the 95% ethanol extract of *Taraxacum officinale* or Orlistat were measured at concentrations of 250, 125, 100, 25, 12.5 and 4 μg/ml. □, Orlistat; ◆, *T. officinale*. Values represent mean ± SD of triplicate measurements.

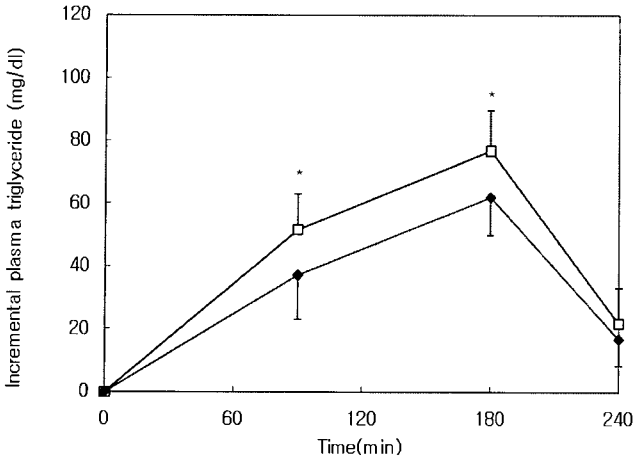

Fig. 2. Increase in plasma triglyceride after administration of *Taraxacum officinale* extract to mice. In the control group (□), corn oil emulsion (5 ml/kg) was administered orally to mice after an overnight fast. In the *Taraxacum officinale* group (◆), corn oil emulsion (5 ml/kg) plus 95% ethanol extract of *T. officinale* (400 mg/kg) was administered orally to mice after an overnight fast. Values represent mean ± SD. *Significantly different at P<0.05

Table 1. Area under the curve (AUC) of postprandial triglyceride responses of mice

Group	AUC (mg · min/dl)
Control group	11,068 ± 2,054
Taraxacum officinale group	8,504 ± 1,950*

Control group: Corn oil emulsion (5 ml/kg) was administered orally to mice after an overnight fast.
Taraxacum officinale group: Corn oil emulsion (5 ml/kg) plus 95% ethanol extract of *T. officinale* (400 mg/kg) was administered orally to mice after an overnight fast.
AUC: Area under the response curves.
Values represent mean ± SD (n=8).
*Significantly different at $p<0.05$

63.0, 56.5, 34.1, 21.9, and 8.1% at concentrations of 250, 125, 100, 25, 12.5 and 4 μg/ml *in vitro*, respectively. Orlistat, a pancreatic lipase inhibitor used as an anti-obesity agent, inhibited the enzyme activity by 95.7 and 90.4% at concentrations of 250 and 4 μg/ml, respectively. The *T. officinale* extract and Orlistat had IC_{50} values of 78.2 μg/ml and 0.22 μg/ml, respectively.

Effect of T. officinale extract on postprandial triglyceride response

Plasma triglyceride responses to a single oral dose of corn oil emulsion (5 ml/kg) alone or the oil emulsion with *T. officinale* extract (400 mg/kg) in ICR mice are shown in Fig. 2. Incremental plasma triglyceride levels of mice that consumed the oil emulsion alone reached a peak (76.9 ± 12.6 mg/dl) at 180 min. Consumption of *T. officinale* extract significantly decreased incremental plasma triglyceride levels at 90 and 180 min ($p<0.05$). The AUC for triglyceride response was significantly lower in the *T. officinale* group (8,504 ± 1,950 mg·min/dl) than in the control group (11,068 ± 2,054 mg·min/dl, $p<0.05$, Table 1).

Discussion

Pancreatic lipase is the key enzyme for dietary fat digestion (Olshansky *et al.*, 2005), and inhibition of the enzyme could be an effective way to alter fat absorption. In fact, Orlistat, pancreatic lipase inhibitor and Sibutramine, an appetite suppressor are the only two anti-obesity medications currently approved (Jandacek & Woods, 2004). However, because Orlistat can result in undesirable side effects, such as fecal incontinence, flatulence, and steatorrhea, its use may be limited (Birari & Bhutani, 2007; Weigle, 2003). Therefore, it may be worthwhile to search the natural substances that show potent inhibitory activity against pancreatic lipase and have fewer side effects. *Alpinia officinarum* (Shin *et al.*, 2003), grape seed extract (Moreno *et al.*, 2003), green tea extract (Juhel *et al.*, 2000), mango leaf and stem bark extracts (Moreno *et al.*, 2006), and onion skin (Kim, 2007) showed potent inhibitory activity against pancreatic lipase.

In this study, we investigated the inhibitory activity of *T. officinale* against porcine pancreatic lipase *in vitro*. At a concentration of 250 μg/ml, the *T. officinale* extract exhibited 90.2% of the inhibition activity of Orlistat against pancreatic lipase, and *T. officinale* inhibited pancreatic lipase in a dose-dependent manner with an IC_{50} of 78.2 μg/ml (Fig. 1). Orlistat showed IC_{50} of 0.22 μg/ml Orlistat was reported to show IC_{50} of 0.14 μg/ml (Weibel *et al.*, 1987). We determined the effect of *T. officinale* on postprandial blood triglyceride in mice after consumption of corn oil emulsion load. The *T. officinale* extract (400 mg/kg) significantly suppressed incremental plasma triglyceride at 90 and 180 min (Fig. 2). Arai *et al.* (1999) reported that n-hexane fraction isolated from *Eugenia uniflora* extract showed pancreatic lipase inhibitory activity *in vitro* and improving effect on postprandial hypertriglyceridemia in mice administered with corn oil emulsion. Kurihara *et al.* (2003) reported that *Cyclocarya paliurus* (Batal) Iljinskaja extract with pancreatic lipase inhibitory activity inhibited postprandial plasma triglyceride increase at 180 min after administration of lard oil or olive oil in mice without influencing postprandial plasma free fatty acid levels suggesting that utilization of triglyceride was not changed. In this study *T. officinale* extract significantly decreased AUC for the postprandial triglyceride response curve. These data demonstrate that *T. officinale* exerts pancreatic lipase inhibitory activity *in vivo* to decrease postprandial triglyceride levels. AUC after a test meal is extensively used as index of the postprandial triglyceride response (Karamanos *et al.*, 2001). The limitation of this study is that we could not distinguish contribution of inhibition of dietary fat absorption and increase of triglyceride clearance to the reduction of postprandial plasma triglyceride response. Further study to measure the effect of *T. officinale* on postprandial plasma free fatty acid and lipoprotein lipase activity would be necessary to determine the effect on triglyceride clearance.

T. officinale leaf was reported to contain flavonoids such as luteolin (Williams *et al.*, 1996) and it was demonstrated

flavonoids could have pancreatic lipase inhibitory activity (Moreno *et al.*, 2006; Shin *et al.*, 2003; Yamamoto *et al.*, 2000). Further study to identify the active compounds which inhibit pancreatic lipase from *T. officinale* leaf could be valued.

Obesity is associated with increased risks for some chronic degenerative diseases and decreased life expectancy (Mokdad *et al.*, 2003; Olshansky *et al.*, 2005). A combination of diet therapy, exercise, and behavior modification is the standard regimen for the treatment of obesity, and medication therapy can be used in conjunction with such lifestyle changes. Since only a few medications have been approved as effective and safe (Jandacek & Woods, 2004), extensive studies have been carried out to develop alternative anti-obesity drugs. Our data suggest that *T. officinale* has potential to be used as an anti-obesity agent.

In conclusion, *T. officinale* showed strong pancreatic lipase inhibitory activity in *vitro* and in *vivo*. Further studies to elucidate anti-obesity effects of chronic consumption of *T. officinale* and to identify the active components responsible for inhibitory activity against pancreatic lipase are necessary.

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