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SHORT COMMUNICATION

Antihyperlipidemic Compounds from the Fruit of *Piper longum* L.

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A bioassay-guided isolation of an ethanol extract of the fruit of *Piper longum* L. yielded piperlonguminine, piperine and pipernonaline, as the main antihyperlipidemic constituents. They exhibit appreciable antihyperlipidemic activity *in vivo*, which is comparable to that of the commercial antihyperlipidemic drug, simvastatin.
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Keywords: antihyperlipidemic activity; *Piper longum* L.; piperlonguminine; piperine; piperonaline.

INTRODUCTION

At present, statins remain the drug of choice for lowering cholesterol levels (Brown *et al.*, 1978). However, the statins have been known to be associated with undesirable side effects including severe myopathy and memory loss (Wagstaff *et al.*, 2003). These developments have spurred the search for new antihyperlipidemic agents (Cho *et al.*, 2006; Jiao *et al.*, 2007; Kumari *et al.*, 2006; Pengzhan *et al.*, 2003; Ravi *et al.*, 2005; Sajjadi *et al.*, 1998; Sharma *et al.*, 2004; Tiwari *et al.*, 2006). In this regard, there has been no attention paid to the antihyperlipidemic constituents in the fruit of *Piper longum* L., a well-known traditional antihyperlipidemic medicine in China. As part of our studies on antihyperlipidemic agents (Han *et al.*, 2008), we are now pleased to report herein the isolation and identification of the antihyperlipidemic compounds present in the fruit of *Piper longum* L., and the comparison of their bioactivities with commercial simvastatin.

EXPERIMENTAL

Materials and reagents. Dried fruit of *Piper longum* L. was purchased in Huhhot, China. The specimen was identified by literature methods (Committee of Chinese Pharmacopoeia, 2000). HPLC grade methanol was used and other reagents were of analytical grade. NMR spectra were recorded using a JEOL ECP 600 MHz spectrometer.

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Extraction and activity-guided isolation. Dried ground fruit of *P. longum* L. (1 kg) was extracted with ethanol (2 L) by refluxing for 4 h with stirring. The ethanol solution was evaporated to dryness at 45 °C to give a black-colored extract (95 g, 9.5%). The pharmacology showed that the ethanol extract has a remarkable antihyperlipidemic activity at 20 mg/kg BW (body weight) (Table 1). The ethanol extract (10.0 g) was separated by silica gel column chromatography eluting with a hexane–ethyl acetate mixture (3:2, v/v). Fractions were collected according to silica TLC analysis to give five fractions denoted as F1, F2, F3, F4 and F5. The silica gel column was eluted with ethanol (2 l) to collect the most polar F6 fraction. By repeating this procedure, a total of 30.0 g of extract was separated to afford F1 (4.35 g, 14.5% yield), F2 (5.70 g, 19.0%), F3 (2.90 g, 9.7%), F4 (4.12 g, 13.7%), F5 (3.61 g, 12.0%) and F6 (7.71 g, 25.7%) after drying. F4 is the most active fraction at 5.6 mg/kg BW (Table 2).

The daily dose of all fractions was determined using the following equation:

$$\text{Daily oral dose of fraction} = 20\ \text{mg/kg BW} \times (\text{yield of fraction}) \times 2$$

F4 (3.5 g) was further separated by similar chromatographic methods as above yielding two pure compounds and one fraction, namely C1 (0.64 g, 2.5% based on the ethanol

Table 1. Effects of the ethanol extract of fruit of *Piper longum* L. on serum total cholesterol (TC) and triglyceride (TG) of rats *in vivo* (mean ± SEM, $n = 10$)

Group	Daily dose (mg/kg B.W.)	TC (mmol/L)	TG (mmol/L)
Normal	–	1.99 ± 0.07^a	0.65 ± 0.06^b
Control	–	7.56 ± 0.87	0.70 ± 0.11
Ethanol extract	20	5.85 ± 0.45^a	0.63 ± 0.10^b

Medicine was orally administered for 15 days.
[a] $p < 0.05$ significant and [b] $p > 0.05$ not significant from control animals (Student's *t*-test).

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Table 2. Effects of the ethanol extract and the fractions from F1 to F6 on serum total cholesterol (TC) and triglyceride (TG) of rats *in vivo* (mean ± SEM, *n* = 10)

Group	Daily dose (mg/kg BW)	TC (mmol/L)	TG (mmol/L)
Control	–	7.40 ± 0.80	0.65 ± 0.05
Ethanol extract	20	5.89 ± 0.82[a]	0.61 ± 0.07[b]
F1	5.8	7.45 ± 0.67[b]	0.66 ± 0.06[b]
F2	7.6	6.98 ± 0.75[b]	0.62 ± 0.08[b]
F3	3.9	5.82 ± 0.68[a]	0.63 ± 0.07[b]
F4	5.6	5.18 ± 1.07[a]	0.50 ± 0.05[a]
F5	4.8	6.61 ± 0.97[b]	0.67 ± 0.07[b]
F6	10.3	6.97 ± 0.89[b]	0.65 ± 0.08[b]

Medicines were orally administered for 15 days.
The daily dose of all fractions was determined using the following equation:
Daily oral dose of fraction = 20 mg/kg BW × (yield of fraction) × 2.
[a] $p < 0.05$ significant and [b] $p > 0.05$ not significant from control animals.

extract), C2 (0.40 g, 1.6%) and F7 (2.14 g, 8.4%). F7 (0.50 g) was separated using reverse phase HPLC (C18 column) eluted with a gradient mixture of methanol and water, starting with 80:20 to pure methanol. The main constituent C3 (0.38 g, 6.4%) was obtained as brown crystals. Pure compounds C1–C3 have very good lipid modulating activities at 5.6 mg/kg BW (Table 3), which is comparable to that of simvastatin at the same dose. Animals and experimental protocols were similar to those described in our previous paper (Han *et al.*, 2008).

RESULTS AND DISCUSSION

An ethanol extract of the fruit of *P. longum* significantly lowered the serum total cholesterol (TC) ($p < 0.05$, Student's test) and slightly decreased serum triglyceride (TG) at a daily dose of 20 mg/kg BW (Table 1). A bioassay-guided isolation of the ethanol extract indicated that of the six fractions obtained (F1–6), fraction F4 significantly decreased TC and TG at the 5.6 mg/kg BW level (Table 2). The main constituents of F4, purified using silica gel and HPLC, showed that they had appreciable lipid modulating activities at 5.6 mg/kg BW, which were comparable to that of simvastatin (Table 3). The structures of the active principles corresponded to the alkaloids piperlonguminine, piperine and pipernonaline (Fig. 1). Their identification was made possible by comparing their NMR spectra with relevant literature data (Tabuneng *et al.*, 1983; Wu *et al.*, 2004). These three compounds significantly modulated serum TC, TG and high density lipoprotein cholesterol (HDL-C), but slightly decreased low density lipoprotein cholesterol (LDL-C). Simvastatin lowered serum TC and LDL-C significantly but had no significant effect on TG and HDL-C. Although some biological properties of piperlonguminine, piperine and pipernonaline have been disclosed recently (Lee *et al.*, 2005; Park *et al.*, 2007; Yang *et al.*, 2002), their lipid modulating activities were hitherto unknown. The lipid modulating activity data on these three alkaloids can be regarded as reliable, due to the fact that the results on simvastatin were in good accordance with literature reports (Grundy, 1998; Kostner *et al.*, 1989). Thus, piperlonguminine, piperine and pipernonaline modulate significantly several lipid parameters, and may provide leads to potentially new antihyperlipidemic drug candidates.

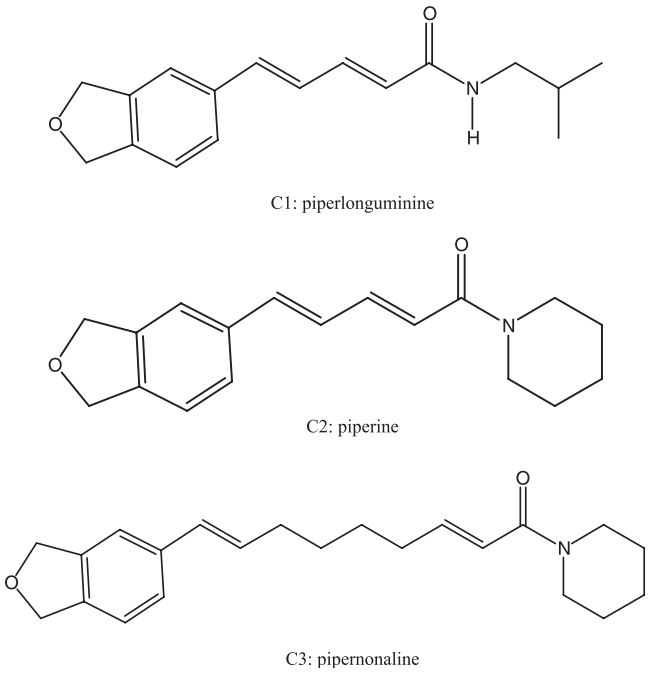

Figure 1. Chemical structures of the three amides.

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Table 3. Effects of C1, C2 and C3, and simvastatin on lipid parameters of rats *in vivo* (mean ± SEM, *n* = 10)

Group	Daily dose mg/kg BW	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	–	7.94 ± 0.84	0.63 ± 0.04	0.57 ± 0.08	4.96 ± 0.54
Simvastatin	5.6	6.27 ± 0.73[a]	0.60 ± 0.05[b]	0.61 ± 0.09[b]	3.55 ± 0.37[a]
C1	5.6	6.06 ± 0.72[a]	0.50 ± 0.06[a]	0.75 ± 0.09[a]	4.51 ± 0.60[b]
C2	5.6	6.25 ± 0.94[a]	0.50 ± 0.08[a]	0.77 ± 0.09[a]	4.62 ± 0.56[b]
C3	5.6	5.95 ± 0.87[a]	0.49 ± 0.09[a]	0.77 ± 0.08[a]	4.55 ± 0.50[b]

C1, piperlonguminine; C2, piperine; C3, pipernonaline; Simvastatin, positive control medicine. Medicines were orally administered for 15 days.
[a] $p < 0.05$ significant (Student's *t*-test) and [b] $p > 0.05$ not significant from control animals.

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