Alcoholic Extract of Lotus Leaves Improves Lipid Profile in Rats with HIV Protease Inhibitor-induced Dyslipidaemia

QJ Su[1], ZZ Lu[1], QY Deng[2], BM Wei[2]

ABSTRACT

Objective: *To examine the effect of the alcoholic extract of lotus leaves (AELL) on antiretroviral treatment-induced dyslipidaemia in a rat model.*

Methods: *Lotus leaves were extracted by 95% ethanol. Seventy male Sprague-Dawley rats were given lopinavir/ritonavir for six weeks. At weeks 0 and 6, sera were collected for measurement of total cholesterol (TC) and triglyceride (TG). Rats meeting the criteria for dyslipidaemia were assigned to four groups and received once daily for another four weeks lopinavir/ritonavir (group A), lopinavir/ritonavir plus 0.52 g/kg AELL (group B), lopinavir/ritonavir plus 0.26 g/kg AELL (group C), or lopinavir/ritonavir plus 0.13 g/kg AELL (group D), respectively. At weeks 8 and 10, blood samples were collected again for measurement of TC and TG.*

Results: *Both TC and TG increased over time in group A during the observation period (weeks 6 to 10), however, TC and TG decreased in group B, and TG declined in group C. Neither TC nor TG could be reduced to a level near baseline.*

Conclusion: *Alcoholic extract of lotus leaves may have the potential to treat dyslipidaemia related to highly active antiretroviral treatment, but may not be potent enough to reduce TC or TG concentrations to goal levels when used alone.*

Keywords: Dyslipidaemia, highly active antiretroviral therapy (HAART), lipid, lotus leaves

El Extracto Alcohólico de las Hojas de Loto Mejora el Perfil Lipídico en Ratas con Dislipidemia Inducida por Inhibidores de la Proteasa del VIH

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RESUMEN

Objetivo: *Examinar el efecto del extracto alcohólico de hojas de loto (EAHL) en la dislipidemia inducida por el tratamiento de antirretrovirales en un modelo de rata.*

Métodos: *De las hojas de loto se extrajo etanol al 95%. Setenta ratas machos Sprague-Dawley recibieron lopinavir/ritonavir durante seis semanas. En las semanas 0 y 6, se recolectaron sueros para medir el colesterol total (CT) y los triglicéridos (TG). Las ratas que satisfacían los criterios diagnósticos de la dislipidemia fueron asignadas a cuatro grupos y recibieron: una vez al día diariamente por cuatro semanas lopinavir/ritonavir (grupo A); lopinavir/ritonavir más 0.52 g/kg EAHL (grupo B); lopinavir/ritonavir más 0.26 g/kg EAHL (grupo C); o lopinavir/ritonavir más 0.13 g/kg EAHL (grupo D), respectivamente. En las semanas 8 y 10, se recogieron muestras de sangre otra vez para medir CT y TG.*

Resultados: *Tanto CT como TG aumentaron con el tiempo en el grupo A durante el período bajo observación (semanas 6 a 10). Sin embargo, TC y TG disminuyeron en el grupo B, y TG disminuyó en el grupo C. Ni TC ni TG pudieron ser reducidos a un nivel cerca de la línea de base.*

Conclusión: *El extracto alcohólico de hojas de loto puede tener el potencial para tratar la dislipidemia relacionada con el tratamiento antirretroviral de gran actividad, pero puede no ser lo suficientemente potente para reducir las concentraciones de TC ó TG a los niveles de los objetivos deseados, cuando se usa solo.*

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Palabras claves: Dislipidemia, terapia antirretroviral de gran actividad (TARGA), lípidos, hojas de loto

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INTRODUCTION

Highly active antiretroviral therapy (HAART) or combined antiretroviral therapy (cART) is a combination of several potent drugs against human immunodeficiency virus (HIV). Despite the clinical benefits, current HAART regimens may have some serious side effects and toxicities. Metabolic disorders, also known as lipodystrophy, including dyslipidaemia, insulin resistance, hyperglycaemia, and abnormal redistribution of body fat, are common problems with AIDS patients receiving HAART (1, 2). These changes increase the risk of developing cardiovascular diseases (3, 4). HIV protease inhibitor (PI)-containing HAART is extremely effective in decreasing viral load and has a profound impact on the clinical history of HIV in patients; meanwhile, it has also been considered as a high risk factor for the development of dyslipidaemia in this population (1, 5–7).

The treatment options for dyslipidaemia in HIV-infected patients include dietary and lifestyle modification, the use of lipid-lowering medications, and switching to a different antiretroviral drug class (8). Lifestyle changes such as smoking cessation, dietary intervention and aerobic physical activity should be introduced to patients (8, 9), but lifestyle changes may have minor effects in reducing lipids to the normal range (10–12). Switching antiretroviral drugs should only be considered when there is a viable alternative antiretroviral regimen, and the new regimen is likely to induce less dyslipidaemia than the original and is likely to possess similar or enhanced antiretroviral potency compared to the original regimen (13, 14). In addition, because of the multifactorial nature of dyslipidaemia in HIV infection, abnormal lipid profile may not be resolved simply by switching drugs (8). The common lipid-lowering agents for general patients, such as fibrates and statins, will reduce serum triglycerides and cholesterol in AIDS patients receiving cART (13, 15, 16). However, potential drug interactions between lipid-lowering agents and antiretroviral agents or other drugs may occur and lead to serious clinical consequences, such as myopathy or rhabdomyolysis (13, 17, 18).

Lotus (*Nelumbo nucifera* Gaertn) is a perennial, rhizomatous, aquatic perennial plant that belongs to the family Nelumbonaceae, and is cultivated mainly in eastern Asia and India (19). Lotus serves as an ornamental plant and its seeds, young stems, and rhizomes are consumed as food. All parts of *N nucifera* have medicinal uses (20). Lotus leaves have been used in traditional medicine to clear heat, resolve summer heat and stop bleeding (21, 22). Previous studies have demonstrated that lotus leaves are effective in hyperglycaemia, dyslipidaemia, and obesity in animal models (23–26), and also in treating dyslipidaemia in human patients (27).

To our knowledge, lotus leaves have not been used in dyslipidaemia related to HAART. In consideration of the highly safe properties of lotus leaves and their potential effect on dyslipidaemia, this research was conducted to evaluate the effects of alcoholic extract of lotus leaves (AELL) in treating dyslipidaemia induced with protease inhibitors (lopinavir/ritonavir) in rats.

SUBJECTS AND METHODS

Plant materials and preparation of plant extracts

The leaves of Nymphaea lotus were collected from Nanning, Guangxi Province of China. The plant leaves were cleaned and dried at 60 ºC to constant weight and then extracted following the procedure as previously described (28). In brief, 500 g of powdered leaves of Nymphaea lotus were soaked in 95% ethanol (2000 mL) for 72 hours and were filtered. The residue was re-extracted under the same conditions. The filtrate was then concentrated under vacuum at 30 ºC. After complete evaporation, the extract was weighed and preserved at 5 ºC. A total of 3000 g lotus leaves was extracted and 384 g products (weighted 12.8% of the dried leaves) were gained.

Animals and study procedure

Seventy male Sprague-Dawley rats weighting 180–220 g were fed with a standard rodent chow containing 4% (w/w) fat and 0.04% (w/w) cholesterol, and given a dose of 250/62.5 mg/kg lopinavir/ritonavir tablet (Kaletra, Abbott Laboratories) suspended in 0.5% sodium carboxymethyl cellulose (CMC) *via* oral gavage once daily. The dose of lopinavir/ritonavir was determined by referring to the previous study (29). At weeks 0 and 6, rats were fasted overnight, with free access to water, and 1 mL of blood samples was collected from the orbital plexus of rats. Sera were isolated and analysed for total cholesterol (TC) and triglyceride (TG) concentrations within six hours using enzymatic kits (Nanjing Jiancheng Bioengineering Institute, China). Dyslipidaemia was defined as an increase of ≥ 30% in TC or TG levels compared to week 0 levels. Rats with dyslipidaemia were randomly divided into four groups using a random number table, and received once daily for another four weeks a dose of 250/62.5 mg/kg lopinavir/ritonavir (group A), 250/62.5 mg/kg lopinavir/ritonavir plus 0.52 g/kg AELL (group B), 250/62.5 mg/kg lopinavir/ritonavir plus 0.26 g/kg AELL (group C), or 250/62.5 mg/kg lopinavir/ritonavir plus 0.13 g/kg AELL (group D). The low dose of AELL (0.13 g/kg) was equivalent to human dose based on the body surface area. The dosage of lotus leaves for human adults is 10 g daily (21). To convert the dose for humans to a dose based on surface area for rats, divide 10 g by 60 kg and then multiply by 6.2 (30, 31). This calculation resulted in a rat equivalent dose of approximately 1 g of lotus leaves per kg body weight, which equates to approximately 0.13 g of AELL per kg body weight. Lopinavir/ritonavir and AELL were suspended in 0.5% CMC. Rats were weighed once a week to adjust drug

intake. At weeks 8 and 10, blood samples were collected again, and sera were isolated and immediately stored at -80 °C until analysis for TC and TG concentrations.

Statistical analysis

Data are expressed as mean ± standard deviation. Differences at each time point among groups were evaluated by one-way analysis of variance (ANOVA). Repeated-measures data were analysed by repeated-measures ANOVA and post-hoc test (Tukey HSD). A two-sided p value ≤ 0.05 was considered statistically significant. Statistics were performed using SPSS version 16.0 (SPSS Inc).

RESULTS

After six weeks of treatment with lopinavir/ritonavir, 51 rats met the criteria for dyslipidaemia. Two rats with lowest TG and one with lowest TC were excluded, and the remaining 48 were selected and randomly assigned into four groups, with 12 rats in each group. Analysis using the one-way ANOVA indicated that TC and TG concentrations did not significantly differ among the four groups at weeks 0 and 6 (Tables 1 and 2).

Analysis of the data obtained from weeks 6 to 10 using the repeated-measures ANOVA showed that both TC and TG concentrations in group A increased over time ($p < 0.001$ and $p < 0.01$, respectively), however, TG in groups B and C and TC in group B declined over time ($p < 0.001$, $p < 0.01$, $p < 0.001$, respectively). Total cholesterol slightly declined in group C but did not reach the level of statistical significance ($p = 0.096$). Both TC and TG did not obviously vary in group D (Tables 1 and 2). The test of between-subject effects of the repeated-measures ANOVA is summarized in Table 3; the result showed that both TC and TG concentrations differed among groups ($F_{(3, 44)} = 2.898$, $p = 0.046$ and $F_{(3, 44)} = 4.192$, $p = 0.011$, respectively).

Table 1: Comparison of total cholesterol concentrations among groups at each time point and among time points within each group

Group	Week 0	Week 6	Week 8	Week 10	F^*	p^*
A	2.70 ± 0.34	3.79 ± 0.39	4.07 ± 0.46	4.24 ± 0.39	62.412	< 0.001
B	2.68 ± 0.39	3.77 ± 0.49	3.51 ± 0.45	3.32 ± 0.50	37.048	< 0.001
C	2.65 ± 0.36	3.78 ± 0.46	3.72 ± 0.46	3.57 ± 0.57	2.850	0.096
D	2.73 ± 0.27	3.81 ± 0.41	3.83 ± 0.47	3.86 ± 0.45	0.170	0.688
F	0.124	0.017	3.052	8.243		
p	0.946	0.997	0.038	< 0.001		

*The statistic was calculated for repeated measures data at weeks 6, 8 and 10, and did not include those at week 0.

Table 2: Comparison of triglyceride concentrations among groups at each time point and among time points within each group

Group	Week 0	Week 6	Week 8	Week 10	F^*	p
A	0.88 ± 0.15	1.53 ± 0.36	1.69 ± 0.29	1.74 ± 0.27	12.960Å	0.003
B	0.88 ± 0.10	1.53 ± 0.16	1.28 ± 0.18	1.14 ± 0.16	38.883	< 0.001
C	0.91 ± 0.09	1.54 ± 0.30	1.45 ± 0.25	1.34 ± 0.25	8.407*	0.008
D	0.87 ± 0.10	1.51 ± 0.24	1.50 ± 0.25	1.52 ± 0.21	0.371	0.694
F	0.226	0.027	5.562	15.237		
p	0.878	0.994	0.003	< 0.001		

*The statistic was calculated for repeated measures data from weeks 6, 8 and 10, and did not include those at week 0.

ÅMauchly's test showed that the data violated the assumption of sphericity, thus a Greenhouse-Geisser correction was made to the degree of freedom for testing the *F* statistic for significance.

Table 3: Test of between-subject effects of the repeated-measures ANOVA

Source	Type III sum of squares	df	Mean square	F	p	Partial eta squared
TC						
Intercept	2049.448	1	2049.448	3631.763	< 0.001	0.988
Group	4.906	3	1.635	2.898	0.046	0.165
Error	24.830	44	0.564			
TG						
Intercept	315.299	1	315.299	1888.679	< 0.001	0.997
Group	2.099	3	0.700	4.192	0.011	0.222
Error	7.345	44	0.167			

ANOVA – analysis of variance; TC – total cholesterol; TG – triglyceride

Table 4: Tukey HSD post-hoc analysis of total cholesterol (TC) and triglyceride (TG) between groups

Group I	Group J	TC			TG		
		Mean difference (I-J)	Std error	*p*	Mean difference (I-J)	Std error	*p*
A	B	0.501	0.177	0.034	0.336	0.096	0.006
	C	0.344	0.177	0.225	0.208	0.096	0.151
	D	0.201	0.177	0.670	0.146	0.096	0.439
B	A	-0.501	0.177	0.034	-0.336	0.096	0.006
	C	-0.157	0.177	0.812	-0.128	0.096	0.551
	D	-0.300	0.177	0.339	-0.190	0.096	0.214
C	A	-0.344	0.177	0.225	-0.208	0.096	0.151
	B	0.157	0.177	0.812	0.128	0.096	0.551
	D	-0.143	0.177	0.850	-0.062	0.096	0.916
D	A	-0.201	0.177	0.670	-0.146	0.096	0.439
	B	0.300	0.177	0.339	0.190	0.096	0.214
	C	0.143	0.177	0.850	0.062	0.096	0.916

Further analysis by Tukey HSD post-hoc test showed that group A had higher TC and TG levels than group B ($p < 0.05$ and $p < 0.01$, respectively). No significant differences were seen in other pairwise comparisons (Table 4).

DISCUSSION

Dyslipidaemia in HIV/AIDS patients may be caused by HIV itself, by antiretroviral drugs, or by host factors (32). Dyslipidaemia may worsen the situation of patients receiving HAART since it has been found to be associated with increased cardiovascular events (7, 33–35). It is reported that up to 81.5% of patients on HAART for at least 12 months develop dyslipidaemia (36). Specialists have suggested that treatment should be instituted for those with previous cardiovascular disease or a high risk of cardiovascular disease (13). However, the treatment of dyslipidaemia is often complicated by potential drug interactions between lipid-lowering agents and antiretroviral agents or other drugs used in the treatment of HIV infection such as antibiotics (13). Although the underlining mechanisms of drug interactions have not yet been clearly elucidated, scientists have suggested that antiretroviral agents may competitively inhibit the CYP3A4 metabolism of lipid-lowering agents; in addition, organic anion transporting polypeptide C (OATP-1B1), apolipoprotein C-III, and sterol-regulatory element-binding protein (SREBP)-1c may also be involved (37–39). Therefore, lipid-lowering agents are required to be more carefully administered to patients on HAART compared with the general population (36). Although a few lipid-lowering drugs such as gemfibrozil, atorvastatin or rosuvastatin are relatively safe and potent for treatment of HIV-infected patients with dyslipidaemia (40, 41), they are relatively expensive for most patients in developing countries, especially those from rural areas. All of these suggest that further efforts are needed to develop effective, safe and inexpensive alternatives for HIV/AIDS patients with dyslipidaemia.

In the present study, we determined the hypolipidaemic activity of AELL in a rat model of HIV PI-induced dyslipidaemia. The results showed that high-dose AELL could decrease serum TC and TG concentrations in rats with PI-induced dyslipidaemia. Middle-dose AELL could significantly decrease TG but only cause a slight decrease in TC, implicating that AELL may have a greater effect on TG than on TC. Although low-dose AELL could not decrease TC and TG, it was obvious that TC and TG levels in rats on low-dose AELL have remained roughly stable. On the other hand, neither TC nor TG concentration could be reduced to the level near to its baseline, even at a high dose of AELL, suggesting that AELL used alone may not be potent enough to reduce TC or TG concentrations to the goal levels, and that the active ingredients of AELL may need to be further purified and concentrated to a more appropriate concentration with stronger effects.

Combined gas/liquid chromatography-mass spectroscopy has shown that lotus leaves are rich in alkaloids and flavonoids (20, 42), both of which have been found to be associated with lipid-lowering and anti-obesity effects (43, 44). Furthermore, several isolates belonging to benzylisoquinoline alkaloids or flavonoid glycoside, gained from further partition of total alkaloids and flavonoids, were found to display significant anti-HIV activity (22). These findings suggest that the extracts of lotus leaves may have the potential to be used in HIV/AIDS patients with dyslipidaemia for two purposes, lowering lipids and inhibiting HIV.

In summary, we found that the alcoholic extract of lotus leaves decreases TC and TG concentrations in rats with dyslipidaemia induced by lopinavir/ritonavir, suggesting that it may have the potential to treat dyslipidaemia related to HAART. However, it may not be potent enough to reduce TC or TG concentrations to the goal levels when used alone.

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