Hypolipidemic activity of *Polygonatum alte-lobatum* Hayata extract in hamsters with hyperlipidemia induced by high-fat diet

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Abstract: This study was to evaluate the hypolipidemic activity of *Polygonatum alte-lobatum* Hayata extract (PAHE) in hamsters fed with high-fat diet (HFD). Experimentally induced hyperlipidemia was produced by feeding hamsters with HFD for 14 days. Hypercholesterolemic hamsters were administered gavagely with PAHE at the dose of 300 and 750 mg/kg bw along with HFD for 28 days in order to estimate their hypolipidemic activity. The lipid profile and histopathological studies were carried out at the end of experiment. Supplementation with PAHE resulted in hypolipidemic effect by lowering the serum lipid parameters such as significant decrease in total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL), and increase in high-density lipoprotein cholesterol (HDL). Significant decrease of TC and triglyceride (TG) was also found in liver after administration of PAHE for 28 days. Histopathological findings in hamster liver supported the effect of PAHE on reduction of HFD-induced hepatic steatosis. The consumption of *P. alte-lobatum* may act as a functional food with ameliorating hyperlipidemia.

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1. Introduction

Cardiovascular diseases are leading cause of death in both industrialized and developing nations（1）. Hyperlipidemia is considered to be a major risk factor for cardiovascular diseases including atherosclerosis, myocardial infarction, heart attacks, and cerebrovascular diseases（2,3）. Hyperlipidemia is characterized by elevated serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) with decreased high-density lipoprotein cholesterol (HDL) levels（4）. The known lipid lowering drugs, such as fibrates, statins and bile acid sequestrants have many side effects in patients（5,6）. Thus, natural plant products have been applied as one of the most attractive sources for medicinal purposes including hypolipidemic drugs in the recent years.

Polygonatum alte-lobatum Hayata belongs to the family of Liliaceae and is a rhizomatous perennial herb. The mature rhizomes are branched with a yellow to green color. In Taiwan, *P. alte-lobatum* is a Formosan endemic plant and has been used as a tonic herbal drug（7）. Recently, it is also being used as one of the components in a herbal drink in Taiwan. In case of components research, two new homologous series of 1,4-benzoquinones and 13 known compounds were isolated and characterized from the rhizomes of *P. alte-lobatum*（7）. However, little information has been obtained on its pharmacological activities after literature survey. This study was to investigate the hypolipidemic activity of *P. alte-lobatum* extract in hamsters with hyperlipidemia induced by high-fat diet (HFD).

2. Material and Methods

Material

The rhizomes of *P. alte-lobatum* were collected from Nantou County, Taiwan. The rhizomes of *P. alte-lobatum* were authenticated by Mr. Huang at the Medicinal Plant Research Laboratory, Department of Pharmacy, Tajen University (Pingtung, Taiwan). A voucher specimen (TU-LFA-100001) was deposited in our research laboratory for future reference. Biochemical kits for TG (GPO-PAP), TC (CHOD-PAP) and HDL were purchased from Fortress Diagnostics Limited (Antrim, UK). Bradford reagent for total protein assay was from Sigma (St. Louis, USA). Normal Diet (ND) and HFD (2% cholesterol enriched diet) were the products of LabDiet and TestDiet (Richmond, USA), respectively.

Preparation of *P. alte-lobatum* H. extract (PAHE)

Fresh rhizomes of *P. alte-lobatum* were cut into small pieces and air dried in the shade. The pulverized powder of *P. alte-lobatum* were extracted with 50 % ethanolic solution for 3 h in a reflux extraction apparatus (Angu, Kaoshiung, Taiwan). After that, the extract solution was filtered using filter paper and filter funnel. The filtered extract mixed with excipient (extract : starch= 1 : 1) was completely dried by a rotary evaporator (Buchi, Flawil, Switzerland). The obtained PAHE was stored in an electronic dry cabinet (Komry, Taipei, Taiwan) for following study.

Animals

Syrian hamsters were bought from BioLASCO Taiwan Co. Ltd. Animals were maintained under standard laboratory conditions (12 h light/dark cycle, temperature (22 ± 2) ℃). Hamsters were procured 1 week before the experiments to allow them to acclimatize to the laboratory environment. Standard chow and water were available *at libitum*. This study was approved by the appropriate animal care and use committee of Tajen University with approval No. IACUC-100-36.

Experimental design for hypolipidemic activity of PAHE

In accordance with the various treatments, animals (5 weeks old) were randomly divided into four groups of five hamsters each. Group 1 (Normal control) was served as normal and fed with normal diet throughout the course of study. Experimentally induced hyperlipidemia was produced by feeding hamsters with HFD (2% cholesterol enriched diet) for 14 days in group 2-4. Group 2 (HFD-control group): Fed with HFD and administered gavagely vehicle for 28 days. Afterwards, group 3 (HFD-PAHE300 group): Fed with HFD and administered gavagely PAHE, 300 mg/kg/bw in vehicle for 28 days. Group 4 (HFD-PAHE750 group): Fed with HFD and administered gavagely PAHE, 750 mg/kg bw in vehicle for 28 days.

Biochemical determination of lipid profile

At the end of experiment, the blood samples were withdrawn from the eye vein and transferred directly into centrifuge tubes after an overnight fast and allowed to clot at room temperature for 30 min. The supernatant clear serum was obtained after centrifugation for 20 min at 6000 r.p.m and then transferred carefully with the help of micropipette into small test tubes for estimation. After that, the animals were sacrificed with CO_2 and the livers were removed for biochemical analysis and histopathological studies..

The serum level of TC, HDL and TG were estimated using commercially available kits (Fortress Diagnostics Kits) according to manufacturer's instruction. VLDL level was calculated as TG/5 while LDL level was calculated with the help of the equation as LDL = TC− HDL– (TG/5) （8,9）. The atherogenic index (AI) and % Protection were calculated as AI = (TC-HDL)/HDL and % Protection = (AI of control – AI of tested group / AI of control) × 100 %, respewctively[1]（10,11）.

Histopathological examination

Livers obtained from the sacrificed animals were washed with normal saline solution and immersed in a 10 % buffered formalin solution. The fixed livers were embedded in paraffin wax and processed in a paraffin tissue processing machine (Leica, Nussloch, Germany). Tissue sections of the liver were made at a thickness of 5 μm and stained with hematoxylin and eosin (H&E) for microscopically examination (Nikon, Tokyo, Japan).

Statistical analysis

All the experimental values are presented in the means ± standard deviation (SD). Statistical comparisons were made by one-way ANOVA and subsequently applying Duncan test was performed using a SPSS statistic software, version 10.0 (Illinois, USA). Statistical significance was defined as $p < 0.05$.

3. Results and discussion

The aim of the study was to assess the hypolipidemic activity of rhizomes of *P. alte-lobatum.* Effect of *P. alte-lobatum* H. extract (PAHE) on serum lipid profiles in HFD-induced hyperlipidemic hamsters was carried out. As shown in Table 1, a significant increase in the level of serum TC, TG and VLDL were found in the animals fed HFD and HDL levels were decreased over a period of 28 days, when compared to the group 1 (Normal control). As compared to the HFD hamsters, PAHE (300 and 750 mg/kg/ bw) showed significant hypolidemic effect by lowering the serum levels of biochemical parameters including TC and LDL (Table 1), and significant increase in HDL level at dose of 750 mg/kg/ bw (Table 2). In case of liver determination, a significant increase in level of TC and TG was found in HFD-induced hamsters, when compared to the group 1 (normal control). As compared to the HFD hamsters, the supplementation of PAHE (300 and 750 mg/kg bw) to HFD hamsters resulted in significant reduction in level of TC and TG (Table 3).

Table 1. Effect of *Polygonatum alte-lobatum* Hayata extract (PAHE) on serum biochemical parameters in high-fat diet (HFD) induced hyperlipidemic hamsters.

Group	Treatment	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I	Normal control	132.21±13.6	32.16±5.55	56.40±10.23	6.43±1.11

Group II	HFD-Control	203.62±20.36$^{###}$	80.26±3.02$^{###}$	120.37±22.51$^{###}$	16.05±0.60$^{###}$
Group III	PAHE (300 mg/kg)	159.54±8.84***	80.93±2.41	75.81±13.90***	16.19±0.48
Group IV	PAHE (750 mg/kg)	155.08±10.52***	80.96±4.87	60.09±13.21***	16.19±0.97

Values are expressed as mean ± SD (n =5).Values are statistically significant at $^{###}$ P<0.001 vs. normal group; *** P<0.001 vs. HFD-control group, respectively (One-way ANOVA followed by Duncan's test). Parameters: TC-total cholesterol, TG-triglycerides, LDL-low-density lipoprotein cholesterol, VLDL-very low-density lipoprotein cholesterol.

Table 2. Effect of PAHE on serum high-density lipoprotein cholesterol (HDL) and atherogenic index (AI) in HFD induced hyperlipidemic hamsters.

Group	Treatment	HDL (mg/dl)	AI	% Protection
Group I	Normal control	69.37±7.52	0.91±0.18	54.45±9.24
Group II	HFD-Control	67.20±3.11	2.05±0.41$^{###}$	0.00±0.00$^{###}$
Group III	PAHE (300mg/kg)	69.70±6.98	1.35±0.28**	29.91±21.43**
Group IV	PAHE (750mg/kg)	78.80±5.65*	0.98±0.20***	51.00±12.09***

Values are expressed as mean ± SD (n =5). Values are statistically significant at $^{###}$ P<0.001 vs. normal group; *** P<0.001, ** P<0.01, * P<0.05 vs. HFD-control group, respectively (One-way ANOVA followed by Duncan's test).

Table 3. Effect of PAHE on liver level of total cholesterol (TC) and triglyceride (TG) in HFD induced hyperlipidemic hamsters.

Group	Treatment	TC (mg/g tissue)	TG (mg/g tissue)
Group I	Normal control	1.07±0.99	1.49±0.31
Group II	HFD-Control	5.94±1.69$^{###}$	7.24±0.99$^{###}$
Group III	PAHE (300 mg/kg)	3.82±1.80*	6.72±1.80*
Group IV	PAHE (750 mg/kg)	4.30±1.03*	6.71±1.08*

Values are expressed as mean ± SD (n =5). Values are statistically significant at $^{###}$ P<0.001 vs. normal group; * P<0.05 vs. HFD-control group, respectively (One-way ANOVA followed by Duncan's test).

Table 4. Effect of PAHE on liver histopathological evaluation in HFD induced hyperlipidemic hamsters.

Group	Treatment	Histoscore
Group I	Normal control	0.20±0.40
Group II	HFD-Control	4.40±0.49$^{###}$
Group III	PAHE (300 mg/kg)	2.40±0.49***
Group IV	PAHE (750 mg/kg)	2.60±0.49***

Values are expressed as mean ± SD (n =5). Values are statistically significant at $^{###}$ P<0.001 vs. normal group; *** P<0.001 vs. HFD control group, respectively (One-way ANOVA followed by Duncan's test).

Hyperlipidemia contributes significantly in the manifestation and development of atherosclerosis and coronary heart diseases (CHD)〔6〕. The atherogenic index (AI) is a marker of atherogenicity because it is increased in people with higher risk of cardiovascular diseases. AI was applied to evaluate the potential anti-atherogenic of the drugs〔12〕. Significant results in lowering of AI by PAHE were found in Table 2. The PAHE at the doses of 300 and 750 mg/kg bw showed an improvement of the cardio vascular risk level by decrease of AI by 29.91 and 51.00, respectively, when compared to the HFD control group.

Histopathological evaluation of PAHE on liver in HFD-induced hyperlipidemic hamsters was shown in Fig. 1. As shown in Fig.1 (group I), the liver of normal control revealed normal liver histopathologic structure, while the livers presented various degrees of fatty change (steatosis) and necrosis with fibrosis in HFD control (group II) and PAHE at the dose of 300 and 750 mg/kg bw (group III and IV) along with HFD for 28 days. Degree of lesions was graded from one to five depending on severity: 1 = minimal (< 1%); 2: slight (1-25%); 3 = moderate (26-50%); 4 =moderate/severe (51-75%); 5 = severe/high (76-100%). As shown in Table 4, a significant increase in histoscore was found in the animals fed HFD as compared to the normal control (group 1). HFD hamsters resulted in significant reduction in histoscore after supplementation of PAHE (300 and 750 mg/kg bw) for 28 days ($p < 0.001$) as compared to the HFD-control (group 2). These results revealed that the histoscore on histopathological evaluation of liver lesion can be decreased in the presence of PAHE indicating in preventing HFD-induced hepatic steatosis.

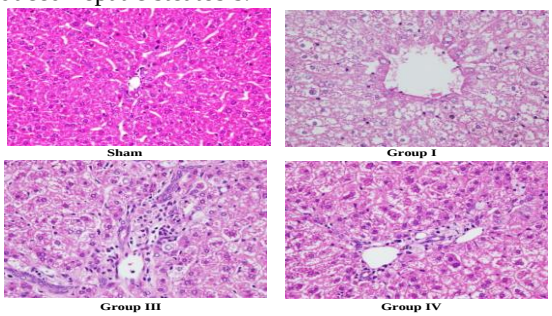

Fig. 1. Histopathological evaluation of PAHE on liver in HFD induced hyperlipidemic hamsters. Group I. normal hamster liver; Group II. HFD treated rat liver; Group III. HFD + PAHE treated rat liver (300 mg/kg

bw); Group IV. HFD + PAHE treated rat liver (750 mg/kg bw). (400 X, H&E stain).

The rhizomes of *Polygonatum* species have been applied as tonic traditional medicines in Asia. It has been reported that *P. odoratum* (Mill.) Druce, *P. falcatum* A. Gray, and *P. sibiricum* Redoute show a hypoglycemic activity, and *P. sibiricum* Redoute exhibits obvious effect in treatment of mouse hypercholesterolemia（13,14）. In case of *P. alte-lobatum*, it is a Formosan endemic plant belonging to *Polygonatum* species and has little information on its pharmacological activities after literature survey. This study is the first report about its cardiovascular pharmacology, and the results show obvious hypolipidemic effect on hyperlipidemia hamsters induced by HFD. Polysaccharides in *P. sibiricum* Redoute has been reported to be responsible for the hypocholesterolemic activity（13）. The polysaccharides in PAHE were analyzed to be 167.87 mg/g. High content of polysaccharides in PAHE could be involved in the biological function of hypolipidemic activity. Several possible mechanisms may be involved in hypolipidemic activities such as reduction of the intestinal absorption of exogenous cholesterol and increase of cholesterol excretion. Further studies on the mechanisms of action are currently under investigation.

In conclusion, we have demonstrated that PAHE possesses hypolipidemic effects on HFD-induced hyperlipidemia in hamsters, which may be associated with polysaccharides in PAHE. Histopathological findings in hamster liver supported the protective role of PAHE in preventing HFD-induced hepatic steatosis. The overall results indicate that *P. alte-lobatum* is a functional food with ameliorating hyperlipidemia..

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References

1. Kumar V., Singh P., Chander R.,F Mahdi., Singh S., Singh R., Khanna A.K., Saxena J.K., Mahdi A.A., Singh V.K. and Singh R.K. (2009). Hypolipidemic activity of *Hibiscus rosa- sinensis* root in rats. *Indian Journal of Biochemistry & Biophysics*. **46(6)**, 507-510.
2. Na Y.Y., Hyeung R.K., Hae Y.C., Jae S.C., (2008). Antihyperlipidemic effect of an Edible brown algae, *Eclonia stolonifera*, and its constituents on Poloxamer 407- Induced Hyperlipidemic and Cholesterol-fed hamsters. *Archies of pharmacal research*. **31(12)**, 1564-1571.
3. Shinde, S., Chivate, N., Kulkarni, P., Naikwade, N. (2013). Hypolipidemic activity of *Psidium Guajava* Linn leaves extracts in hyperlipidemic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*. **5(1)**, 70-72.
4. Kumar, D.S. & Muthu, A.K. (2012). Evaluation of hypolipidemic activity of various extracts from whole plants of *Ionidium Suffruticosum* (Ginc.) (Family: Violaceae) in rat fed with high fat diet. *International Journal of Pharmacy and Pharmaceutical Sciences*. **4(4)**, 69-73.
5. Chattopadhyaya, R., Pathak, D., Jindal, D.P. (1996). Antihyperlipidemic agents. A review. *Indian drugs.* **33**, 85-97.
6. Sowmya, A. & Ananthi, T. (2011). Hypolipidemic activity of *Mimosa pudica* Linn on Butter Induces hyperlipidemia in rats. *Asian Journal of Research in Pharmaceutical Sciences*. **1(4)**, 123-126.
7. Huang, P.l., Gan, K.H., Wu, R.R., Lin, C.N. (1997). Benzoquinones a homoisoflavanone and other constituents from *Polygonatum alte-lobatum*. *Phytochemistry*. **44(7)**, 1369-1373.
8. Sarker, M., Mahmud, Z.A., Saha, S.K., Tithi, N.S., Ali, M.S., Bachar, S.C. (2012). Antihyperlipidemic activity of flowers of *Punica granatum* in poloxamer-407 induced hyperlipidemic mice model. *Pharmacognosy Journal*. **4(27)**, 66-70.
9. Patil, U.K., Saraf, S., Dixit, V.K. (2004). Hypolipidemic activity of seeds of *Cassia tora* Linn. *Journal of Ethnopharmacology*. **90(2-3)**, 249-252.
10. Parab, R.S. & Mengi, S.A. (2002). Hypolipidemic activity of *Acorus calamus* L. in rats. *Fitoterapia*. **73(6)**, 451-455.
11. Neelima, N., Sudhakar, M., Patil, M.B., Lakshmi,B.V.S. (2011). Hypolipidemic activity and HPTLC analysis of *Ixora coccinea* L. Leaves. *Journal of Applied Pharmaceutical Science*. **01 (10)**, 172-175.
12. Pillai, K.K., Chidambaranathan, N., Halith, M.M., Jayaprakash, S., Narayanan, N. (2012). Hypolipidemic activity of ethanolic extract of leaves of *Cnidoscolus Chayamansa* in hyperlipidemic models of wistar albino rats. *Acta Chimica & Pharmaceutica Indica*, **2(1)**, 24-31.
13. Zhang, T.T., Xia, X.K., Chen, C.P., Wu, A.P., He, M., Nie, L.W. (2006). Biological activities of polysaccharides from *Polygonatum Sibiricum* Redoute, *Chinese Journal of experimental traditional medicine formulae.* **12(7)**, 42-45.
14. Ahn, M.J., Kim, C.Y., Yoon, K.D., Ryu, M.Y., Cheong, J.H., Chin, Y.W., Kim, J. (2006). Steroidal saponins from the rhizomes of Polygonatum Sibiricum. *Journal of Natural Products.* **69**, 360-364.