

Original Research Article

Anti-Hyperlipidemic Effect of Flavone-Rich *Belamcanda chinensis* (L.) DC. (Iridaceae) Leaf Extract in ICR Mice Fed High-Fat Diet

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Abstract

Purpose: To assess the anti-hyperlipidemic effect of flavone-rich *B. chinensis* leaf extract (HTP) in ICR mice fed a high-fat diet.

Methods: HTP (100, 200 and 400 mg/kg) were orally administered to ICR mice fed high-fat diet for 7 weeks. Blood glucose, as well as serum and hepatic levels of lipids were determined at the end of the experiment. Phosphorylation of adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) and protein level of peroxisome proliferator-activated receptor α (PPAR α) were analyzed by Western blot and enzyme-linked immunosorbent assay (ELISA) kit, respectively.

Results: Treatment with HTP significantly decreased peri-epididymal fat weight ($p < 0.01$ and $p < 0.05$ for 200 and 100 mg/kg, respectively), lowered serum and hepatic lipid, and decreased glucose area under curve (AUC) in oral glucose tolerance test ($p < 0.01$ for 200 mg/kg). Western blot and ELISA analysis showed that administration of HTP (200 mg/kg) significantly increased AMPK ($p < 0.05$) phosphorylation and PPAR α expression in liver ($p < 0.05$).

Conclusion: HTP can alleviate hyperlipidemia, at least in part, by up-regulation of AMPK and PPAR α .

Keywords: *Belamcanda chinensis*, Flavone, Hyperlipidemia, Adenosine 5'-monophosphate-activated protein kinase, Peroxisome proliferator activated receptor- α , Glucose tolerance

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INTRODUCTION

The incidence of hyperlipidemia is currently increasing at a dramatic rate throughout the world [1]. The close relationship between elevated circulating levels of free fatty acids (FFA), triglycerides (TG) and LDL-cholesterol (LDL-c) and cardiovascular diseases (CVD) has been well documented [2,3] Although current pharmaceuticals such as statins can effectively alleviate hyperlipidemia, they are often

associated with undesirable side effects such as gastrointestinal discomfort, hyperglycemia and myopathy [4,5]. Dietary intervention through intake of polyphenol-rich plant products that can modulate lipid metabolism represents another modality of management [6,7].

AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor α (PPAR α) are key regulators of lipid metabolism. AMPK is activated by phosphorylation at

threonine (Thr172) on the alpha-subunit This in turn promotes ATP-generating mechanisms such as lipid oxidation while decreasing energy-consuming processes like TG and protein synthesis [8,9]. On the other hand, activation of PPAR α by ligands up-regulates the expression of genes involved in fatty acid transport and oxidation [10,11]. AMPK and PPAR α were proposed as major therapeutic targets for hyperlipidemia [12,13].

Belamcanda chinensis belongs to the family of Iridaceae and their rhizomes have been widely used as traditional medicine in China. Many isoflavones such as tectoridin, iridin, irigenin and tectorigenin have been identified from this plant [14]. The antifebrile, antioxidant, anti-inflammatory and hepato-protective activities of *B. chinensis* have been well-documented [15-17]. Previously, we reported the hypoglycemic and anti-hyperglycemic effects of flavone-rich *B. chinensis* leaf extract (HTP) in normal and STZ-induced diabetic rats [18, 19]. Apart from these, no study to-date has investigated the anti-hyperlipidemia activity of HTP. In this study, we assessed the anti-hyperlipidemia effects of HTP on high-fat diet induced obese mice. The stimulating effects of HTP on AMPK and PPAR α were also studied.

EXPERIMENTAL

Plant materials

Leaves of *Belamcanda chinensis* were collected from Hainan province, South China, and authenticated by Dr Hubiao Chen (Health Science Center, Peking University, China). The preparation of HTP was performed as previously reported [18,19]. A voucher specimen has been deposited in School of Life Science, Beijing Institute of Technology (NO. HTP20120911).

Animals and experiment design

Male ICR mice (20 \pm 2 g, Peking University Laboratory Animal Center, Beijing, China) were housed at 22 \pm 2 $^{\circ}$ C and 55 \pm 5 % relative humidity; 12 h light-dark cycle and allowed free access to water and feed. The study was carried out in accordance with International Guidelines for Care and Use of Laboratory Animals [20] and approved by Animal Ethical Committee of Beijing Institute of Technology (reg. no. 201209007/BITAEC).

Seventy-two ICR mice were kept in a humidity-controlled room on a 12-h light-dark cycle with

food and water available ad libitum for one week. Animals were then randomly divided into 6 groups with 12 mice in each group: BC, fed a standard diet and received 0.5 % sodium carboxymethylcellulose (CMC-Na) solution only; NC, fed a high-fat diet (HFD) and received 0.5 % CMC-Na solution only; PC, fed a HFD and treated with gemfibrozil (200 mg/kg); and HTP groups, fed a HFD and treated with HTP (100, 200 and 400 mg/kg, respectively). HFD consists of 10 % lard, 10 % sugar, 10 % egg York, 1 % cholesterol, 0.2 % sodium cholate, and 68.8 % standard diet. HTP and gemfibrozil were administered by oral gavage. Body weight was monitored weekly. During the experiment, blood was collected from tail vein for the measurement of blood glucose. At the end of the 7th week, blood sample was collected from orbital venous plexus and the serum was prepared for biochemical analysis. Liver and peri-epididymal fat were removed, weighted and stored at -70 $^{\circ}$ C refrigerator (Thermo Fisher Scientific, Waltham, USA,). Serum and hepatic levels of triglyceride (TG) and total cholesterol (TCH) were determined by corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instruction. The hepatic PPAR α level was determined by a mouse PPAR- α ELISA kit (HanKe Biotech co., Ltd, Beijing, China) according to the manufacture's instruction.

Oral glucose tolerance test (OGTT)

Animals were orally administered corresponding regents after overnight fast. PC group was treated with acarbose (50 mg/kg). After another 2 h, glucose (2.5 g/kg) was given to each animal orally. Blood were collected from tail vein of each mouse at 0, 30, 60, and 120 min after glucose administration, and glucose levels were determined by a blood glucose meter (Roche Diagnostics, Basel, Switzerland).

Western blot

Western blot analysis was performed on liver tissue extract as previously reported [21]. Antibodies against phospho-AMPK α (Thr172) and AMPK α were from Cell Signaling Technology Inc. (Beverly, USA), and antibody against GAPDH was from Abcam, Inc. (Cambridge, USA).

Statistical analysis

Data are presented as the means \pm S.D. One-way ANOVA was used to determine significant

differences among groups, after which the modified Student's t-test with the Bonferroni correction was used for comparison between individual groups. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of HTP on peri-epididymal fat weight

Animals in all groups showed a steady increase in body weight throughout the experimental period. At the end of the study, the average body weight of NC group was significantly higher than BC group ($p < 0.05$). Treating with HTP for 7 weeks did not influence the weight gain but significantly decreased the peri-epididymal fat weight and fat index (Table 1).

Effect of HTP on blood and liver lipid profiles

In animals fed a HFD (NC group), the serum levels of triglycerides (TG), total cholesterol (TCH), HDL-C, LDL-C and hepatic levels of TG, TCH were all significantly increased as compared with those fed a standard diet (BC group) (Fig. 1). Treatment with HTP (100, 200, 400 mg/kg) or gemfibrozil (200 mg/kg) for 7 weeks significantly decreased the serum and hepatic levels of TG (Fig. 1). HTP did not lower serum TCH but significantly decreased hepatic TCH level at 200 and 400 mg/kg. After 7 weeks of HTP or gemfibrozil treatment, serum HDL-C was increased and LDL-C decreased, but only gemfibrozil-treated group showed significant increase in serum HDL-C level when compared with the vehicle control.

Table 1: Effect of HTP on the body weight, peri-epididymal fat weight and fat index

Group (n=12)	Body weight (g)	Peri-epididymal fat weight (g)	Fat index (mg/g BW)
BC	44.8±4.3	0.40±0.16**	8.14±2.62**
NC	46.1±3.0	0.83±0.30##	18.07±6.74##
PC	43.1±2.3*	0.55±0.18*	12.70±3.84
HTP 100	44.8±3.9	0.59±0.42	9.57±3.15**
HTP 200	44.8±3.6	0.39±0.25**	8.62±5.33**
HTP 400	45.4±2.8	0.57±0.29*	11.13±5.06*

BC = fed a standard diet; NC = fed a high fat-diet (HFD); PC = fed a HFD and treated with gemfibrozil (200 mg/kg); HTPs = fed a HFD and treated with indicated dose of HTP. Values are mean ± S.D.; ## $p < 0.01$ NC vs. BC; * $p < 0.05$, ** $p < 0.01$ vs. NC

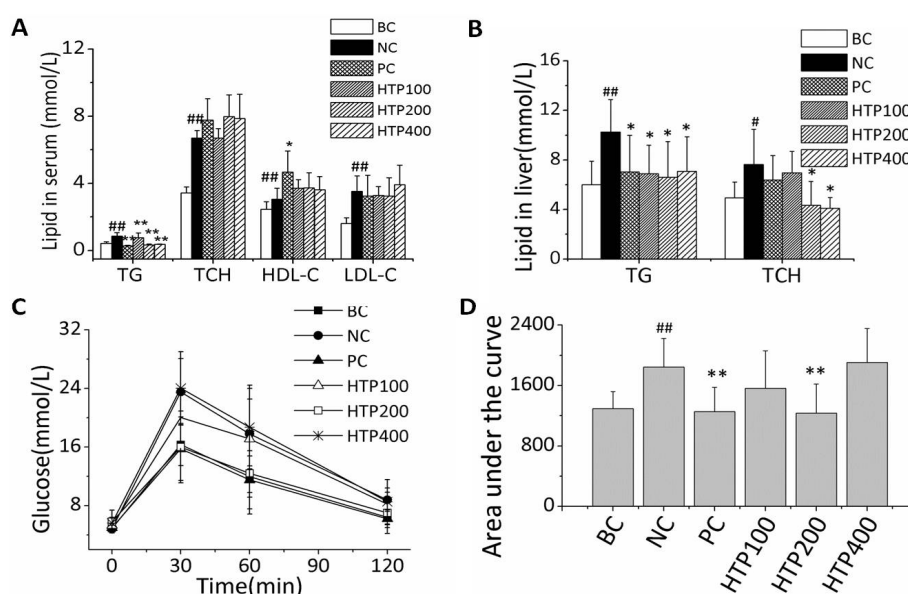


Figure 1: Effects of HTP on serum (A) and hepatic (B) lipid profiles and glucose tolerance (C and D) at the end of 7th week. BC, fed a standard diet; NC, fed a high fat-diet (HFD); PC, fed a HFD and treated with gemfibrozil (200 mg/kg) or acarbose (50 mg/kg for OGTT); HTPs, fed a HFD and treated with indicated dose of HTP. Values are mean ± S.D. # $p < 0.05$, ## $p < 0.01$ NC vs. BC; * $p < 0.05$, ** $p < 0.01$ vs. NC

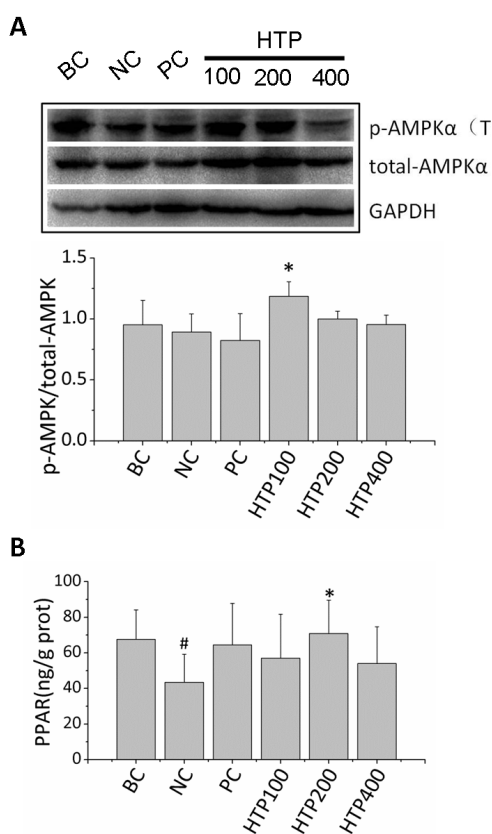


Figure 2: Effect of HTP on AMPK phosphorylation (A) and PPAR α expression (B) in the liver of ICR mice. BC, fed a standard diet; NC, fed a high fat-diet (HFD); PC, fed a HFD and treated with gemfibrozil (200 mg/kg); HTPs, fed a HFD and treated with indicated dose of HTP. Values present as mean \pm S.D.; # $p < 0.05$ NC vs. BC; * $p < 0.05$ vs. NC

Effect of HTP on AUC in OGTT

In oral glucose test (OGTT), acarbose-treated group (PC) and HTP200 group showed significant reduction in blood glucose levels at 30, 60 and 120 min (HTP200: $p < 0.01$; PC: $p < 0.001$). The area under the curve (AUC) of PC group and HTP200 group was significantly lower than that of NC group ($p < 0.01$) (Fig.1).

Effect of HTP on AMPK phosphorylation and PPAR α expression

To ascertain the potential mechanism of HTP-mediated anti-hyperlipidemic effect, the levels of AMPK α and its phosphorylated form (p-AMPK α Thr172) in liver were measured by western-blot analysis. As shown in Fig. 2, the expression level of p-AMPK α (Thr172) in HFD-fed mice was lower than that in standard diet-fed animals. Treatment

with HTP up-regulate the expression of p-AMPK α (Thr172) ($p < 0.05$). ELISA analysis showed that HTP also increased the expression of PPAR α in the liver (Fig. 2).

DISCUSSION

Belamcanda chinensis is a traditional Chinese medicine used for the treatment of cough and pharyngitis. In Hainan Province, South China, the local residents take the decoction of *Belamcanda chinensis* leaves as a folk medicine to cure diabetes-related hyperlipidemia. Our previous study has demonstrated the hypoglycemic activity of total flavones from *Belamcanda chinensis* leaves (HTP) (18,19). Five compounds have been identified in HTP which are 2''-O-rhamnosyl swertisin, swertisin, tectoridin, iristectoriginin A, and iridin [19,22]. Previous studies [23,24] have demonstrated the hypolipidemic and anti-dyslipidemic activity of flavonoid-rich extract, revealing a potential role of flavonoids in the treatment of hyperlipidemia. Lee *et al* [25] and Xiong *et al* [26] have reported the hypolipidemic effects of tectorigenin and tectoridin isolated from *Pueraria thunbergiana* and *Pueraria lobata*, respectively. These two flavonoids also exist in *B. chinensis* leaves [19]. Apart from these, no direct evidence for the anti-hyperlipidemic activity of *B. chinensis* leaf extract has been published up to now. In this work, we demonstrated for the first time that the *B. chinensis* leaf extract (HTP) possessed potent antihyperlipidemic activities in ICR mice fed a HFD.

Our results presented in this work showed that HTP can alleviate hyperlipidemia from several aspects. Firstly, treatment with HTP did not influence the body weight gain but significantly decreased peri-epididymal fat weight. This beneficial effect of HTP was not due to the decrease of food intake as HTP-treated mice showed a similar food intake in our experiment (data not shown). Secondly, treatment with HTP significantly improved the lipid profiles in serum and liver. It preferred decreasing the levels of triglyceride in both serum and liver. Finally, HTP also remarkably decreased the postprandial hyperglycemia, reducing the glucose AUC after OGTT, which suggested the beneficial effect of HTP on insulin sensitivity.

AMPK and PPAR α have been broadly reported to be potential therapeutic target of fenofibrate for the treatment of dyslipidemia [27]. In order to clarify the potential mechanism under HTP-mediated antihyperlipidemic effect, the influence of HTP on the expression of these two key lipid

metabolic regulators was investigated. Our data showed that administration of HTP significantly increased the expression levels of phosphorylated AMPK (p-AMPK α) and total PPAR α in the liver of mice. These results suggested that HTP may exert antihyperlipidemic activity through, at least in part, upregulation of AMPK and PPAR α .

CONCLUSION

The results presented in this study suggest that the flavone-rich *B. chinensis* leaf extract (HTP) has good potentials for lipid management. Up regulation of AMPK and PPAR α are two possible mechanisms for its antihyperlipidemic activity.

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