บรรณานุกรม

ฐานข้อมูลสรรพคุณที่สำนักงานคณะกรรมการอาหารและยาอนุญาตให้ขึ้นทะเบียนยาแผนโบราณ http://www.medplant.mahidol.ac.th/index.asp

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Output ที่ได้จากโครงการ

1. สร้างองค์ความรู้ใหม่เกี่ยวกับฤทธิ์ของ piperine ในการลดปัจจัยเสี่ยงของการเกิดโรคในระบบหัวใจร่วมหลอดเลือด
2. นำเสนอผลงานในที่ประชุมวิชาการระดับชาติ Poster presentation ในที่ประชุมสรีรสมาคมแห่งประเทศไทย 2-4 พฤษภาคม 2555
3. ตีพิมพ์ผลงานระดับชาติ

ภาคผนวก

Manuscript (Accepted and published in Journal of Physiological and Biomedical Sciences)

Piperine Is Antihyperlipidemic And Improves Endothelial-dependent Vasorelaxation In Rats On A High Cholesterol Diet

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Introduction. Piperine is a major ingredient of black pepper and long pepper, which are widely used as a spice and in Ayurvedic medicine. As an anti-hyperlipidemic, some reports show clear blood lipid reductions whilst others failed to show any effect. Therefore, we aimed to resolve this discrepancy and to show whether piperine could improve vascular endothelial function in cholesterol fed rats.
Methods. Male Sprague-Dawley rats (180-250g) were made hypercholesterolemic by daily intragastric gavage of emulsified cholesterol for 8 weeks and piperine was given 8 hr after cholesterol as appropriate to prevent digestive/absorptive interactions. Animals were divided into 4 groups: (i) sham (control), (ii) cholesterol (HC) (iii) the cholesterol plus 40mg/kg piperine (Pip40), and (iv) cholesterol plus 80mg/kg piperine (Pip80). Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were measured at week0 and week8. At week8, rats were killed and endothelium-dependent vasorelaxation induced by acetylcholine in isolated aortic rings.
Results. Throughout the 8 week trial, treatment with piperine (40, 80mg/kg) reduced body weight gain and food intake per day compared with control. The HC group exhibited elevation of both TC and TG. Piperine at 80mg/kg but not low dose (40mg/kg) partially reduced TC, while both doses effectively normalised the elevated TG. HDL was decreased in all animals including controls. Hypercholesterolemic and hypertriglyceridemic rats showed significant reduction of acetylcholine-induced vasorelaxation of isolated aortae and this was prevented by treatment with piperine.
Conclusion. This study showed that piperine reduced body weight gain, lowered TC and fully normalised TG and endothelial-mediated vasorelaxation of aorta. Thus piperine could provide beneficial effects in weight control, antihyperlipidemia and counteracted the poor vascular endothelial function in hyperlipidemia.
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Introduction

Black pepper (*Piper nigrum*) and long pepper (*Piper longum*) have been widely used as spices and in Ayurvedic medicine mainly for its actions on the gastrointestinal tract. Their major active constituent, piperine, possesses various pharmacological actions including antioxidant (1, 2, 3), anti-inflammatory (4, 5), and anti-hypertensive (6) effects. Previous studies also showed it to reduce obesity and hyperlipidemia (7, 8, 9, 10), major risk factors for cardiovascular disease which has become global health problem. However, the findings for rodents are controversial: some studies reported that supplementing piperine with high fat diet reduced body weight, total cholesterol (TC) and triglycerides (TG) (8, 10). In contrast, others demonstrated that a high carbohydrate, high fat diet with piperine produced no effect on both plasma TC and TG (9). These discrepancies are likely to be due to differences in experimental protocol including method of administration.

A consistent pathology resulting from hypercholesterolemia is endothelial dysfunction (11). Thus, we hypothesize that if piperine could normalise blood cholesterol, endothelial function would benefit. The present study was designed to demonstrate a hypolipidemic effect of piperine given as daily bolus doses separate from cholesterol. Such a protocol reduces the confounding influences that piperine might have on the utilisation of the normal diet. After 8 weeks on the diet, the endothelial-dependent vasorelaxant responses were characterised.

Methods

Animals

Male Sprague-Dawley rats (180-250g) were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhorn Pathom, Thailand and maintained under standard conditions: 25±2°C, 12 hours light-dark cycle and received tap water and commercial rat diet *ad libitum*. Experimental protocols were approved by the Animal Ethics Committee, Naresuan University, Phitsanulok, Thailand.

Experimental design

To induce hypercholesterolemia, a freshly prepared cholesterol emulsion (12) was which comprised cholesterol 1500mg (Carlo Erba Reactifs, Italy), bile extract 750mg (Sigma Chemicals, MO, USA), coconut oil 750mg (PN Natural Products, Thailand) and distilled water 3ml. The given dose of cholesterol in each animal was 1500mg/kg/ administered every morning for 8 weeks to each animal, except controls, as single intragastric feedings. Piperine (Sigma Chemicals) was also given by gavage 8 hr after the cholesterol to prevent piperine interfering with absorption.

Animals were divided into 4 groups: (i) sham (control, n=6), (ii) cholesterol (HC, n=8) (iii) cholesterol plus 40mg/kg piperine (Pip40, n=8), and (iv) cholesterol plus 80mg/kg piperine (Pip80, n=6). Piperine at 40mg/kg was previously shown to have an antihyperlipidemic effect (7), thus 40 and 80mg/kg were chosen in this study in order to evaluate its dose dependency. Body weight and food consumption were recorded daily, while the lipid profile was measured at week0 and week8. At week8, rats were anesthetized (Nembutal 20mg/kg, ip injection) and killed by exsanguination.

Lipid profile measurement

The rats were fasted overnight and then ~0.5ml of blood collected from the tail vein (12). Serum samples were centrifuged (7000g, 10min). Serum lipid levels of TC, HDL and TG were determined using a commercial kit (HUMAN Gesellschaft für Biochemica und Diagnostica GmbH, Germany).

Vasorelaxant activity

Immediately after, the rats were euthanised, the thoracic aorta was rapidly removed and cleaned of surrounding loose connective tissue and cut into rings 2-5 mm in length for tension measurements. The rings were mounted on a pair of wires in organ baths containing aerated Krebs' solution (mM): NaCl, 122; KCl, 5; [N-(2-hydroxyethyl) piperazine N'-(2-ethanesulfonic acid)] (HEPES), 10; KH_2PO_4, 0.5; NaH_2PO_4, 0.5; $MgCl_2$, 1; glucose, 11; and $CaCl_2$, 1.8 (pH 7.3), at 37 °C and bubbled with air. The vessel segments were allowed to stabilise for 1 hr at a resting tension of 1 g during which time the solution was refreshed every 15 min. Following stabilisation, acetylcholine (Ach, 0.01-10μM) induced endothelial mediated-relaxations were evaluated in phenylephrine (10μM) pre-contracted arterial rings. Changes in isometric tension were measured using a force transducer (CB Sciences Inc., Milford, USA) connected to a MacLab A/D converter (Chart V5), stored and displayed on a personal computer.

Statistical analysis

All data are expressed as mean ± standard error of mean (SEM) of *n* animals. Statistical significance was assessed using paired or un-paired Student's *t*-test or analysis of variance (ANOVA) followed by Tukey's test, as appropriate. In all comparisons, *p* values less than 0.05 were accepted as significant.

Results

Effect of piperine on body weight and food intake

Control animals progressively gained weight from 243.3±7.6 to 444.8±10.9g (mean±SDs, n=6) throughout the 8 week trial, but all the treated rats showed a lower weight gain compared to controls (p<0.01 for HC (n=8), <0.001 for HC+Pip40 (n=8) and <0.01 for HC+Pip80 (n=6), Figure 1A). For group HC+Pip80 the weight reduction was more pronounced than the HC and HC+Pip40 groups (p<0.05, Figure 1A). The food intake per day reduced in all treatment groups compared to control (Figure 1B). Piperine at 40mg/kg appeared to cause greater effect than other treatments.

Piperine improved the lipid profile

Oral administration of cholesterol produced a clear increase of both serum TC (from 89.6±3.4 (week0) to 148.3±8.8 (week8) mg/dL, p<0.001) and TG (from 76.2±5.7 (week0) to 124.9±11.1 (week8) mg/dL, p<0.01, Table 1). Serum TC was increased (68%) but with piperine at 80mg/kg, this effect was smaller (to 45%), while 40mg/kg was apparently ineffective. For triglycerides, both low (40mg/kg) and high doses (80mg/kg) of piperine normalised the elevated serum TG (Table 1). HDL was consistently decreased across all groups of animal studied (Table 1).

Piperine improved vasorelaxation

Rats given cholesterol showed a reduced Ach-relaxations of isolated aorta (p<0.01 compared to controls, Figure 2). Thus the % maximum relaxation was decreased from 100% to 84.7±3.2% (HC) of controls but the ID_{50} was unaffected. Piperine at both low (40mg/kg) and high (80mg/kg) doses normalized the vasorelaxation of the aorta (p<0.05 compared to HC, Figure 2).

Discussion

The present study showed that dosing with piperine for 8 weeks following a cholesterol gavage slowed the gain in body weight, partially reduced serum TC and completely restored serum TG. Similar findings have been reported by previous studies using hyperlipidemic rat model by Shah et al (10) who demonstrated that piperine reduced body weight, TC, TG, and fat mass, but not food intake in obesity-induced dyslipidemia in high-fat diet rats. This accords with the reduced adipose tissue mass with dietary piperine in rats (10). In contrast, rats consuming piperine in the food showed increased food consumption (9). Thus the difference might arise from gustatory drive.

Piperine may modulate cholesterol absorption (13). However, in the present study the cholesterol was administered 8 hr before the piperine by which time the cholesterol will have been absorbed. Thus, piperine is likely to be acting beyond the absorptive phase. Because of its structural similarity with a melanocortin (MC)-4 agonist, it was previously suggested that piperine might activate hypothalamic MC-4 receptors thus leading to decreased appetite and increase insulin sensitivity (10). Other possible mechanisms of anti-obesity and lipid lowering effects of piperine might involve (i) the inhibitory action of piperine on cholesteryl ester (CE) synthesis, (ii) lipid and lipoprotein accumulation by modulating lecithin cholesterol transferase, lipoprotein lipase and acyl-CoA cholesterol acyltransferase (ACAT) (9) and (iii) anti-inflammatory actions (4, 5).

In the present study, HDL was decreased across all animal groups. The effects on HDL were ambiguous but there is an increasing realization that statins are also inadequate in high risk CVD patients who require additional therapy to raise HDLs (14). Clearly, piperine would need similar supplementation.

It is well known that hyperlipidemia plays a crucial role in endothelial dysfunction. Several lines of evidence demonstrated the impairment of endothelial-mediated vasorelaxation in dyslipidemic animal models (9, 15, 16), which is in agreement with our findings. In the present study, treatment with piperine successfully restored the vasorelaxant function of aorta, consistent with the previous report (9). Apart from its anti-hyperlipidemia, several other mechanisms of action of piperine could be involved in vascular function improvement including: (i) piperine inhibited macrophage ACAT to decrease CE synthesis leading to a reduction of lipid droplets and foam cell formation, which prevent endothelial dysfunction (3), (ii) piperine possessed antioxidant activity, lowered lipid peroxidation, increased glutathione peroxidase and superoxide dismutase activity, thus protecting against oxidative damage of endothelial cells (1, 2, 3) and (iii) piperine inhibits expression of cell adhesion molecules and tumor necrosis factor-α induced adhesion of neutrophils to endothelial cells, thus preventing or delaying the inflammatory process (4, 5). Evidently, piperine has multiple actions but at this stage, clinical studies are needed to determine the effect(s) which have some impact on human disease.

Conclusion

We conclude that piperine reduced body weight gain, lowered TC and fully normalised TG, restored endothelial-mediated vasorelaxation of aorta and acts in the post-absorptive stage. Thus piperine could provide beneficial effects in weight control, anti-hyperlipidemia and vascular endothelial function.

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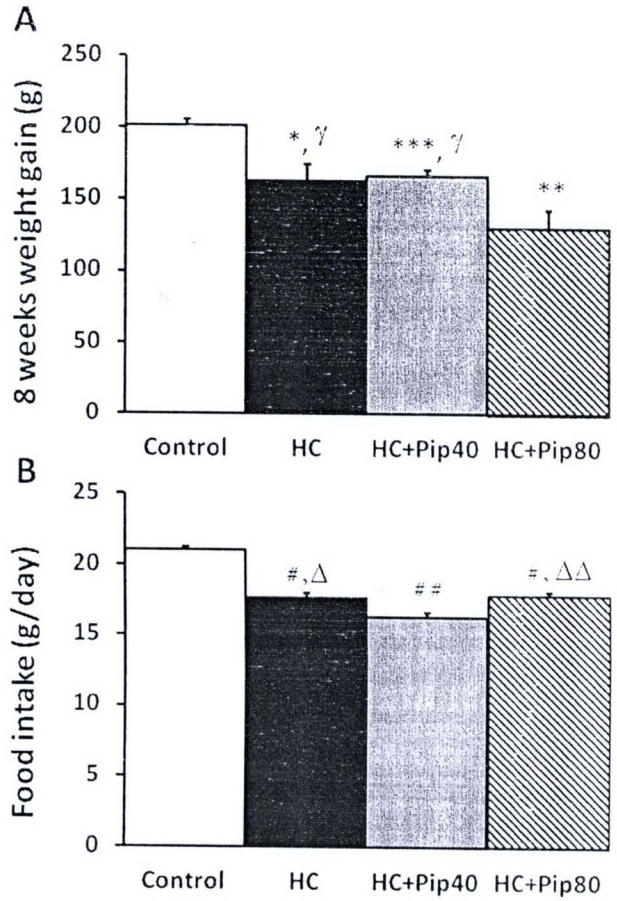

Figure 1

Effect of piperine on (A) weight gain (comparing week0 and week8) and (B) food intake (the averaged daily intake during the 8 week period). Rats were fed daily with water (control), cholesterol (HC) or cholesterol with either 40mg/kg piperine (Pip40), or 80mg/kg piperine (Pip80) as indicated. All points are means±SEMs of all animals from groups as indicated (n=6-8). P-values are indicated as $^{*}p<0.01$, $^{**}p<0.001$, $^{***}p<0.0001$ compared to control; $^{\gamma}p<0.05$ compared to HC+Pip80; $^{\#}p<0.00001$, $^{\#\#}p<0.000001$ compared to control; $^{\Delta}p<0.05$, $^{\Delta\Delta}p<0.01$ compared to HC+Pip40. All data points are mean±SEM (n=6-8).

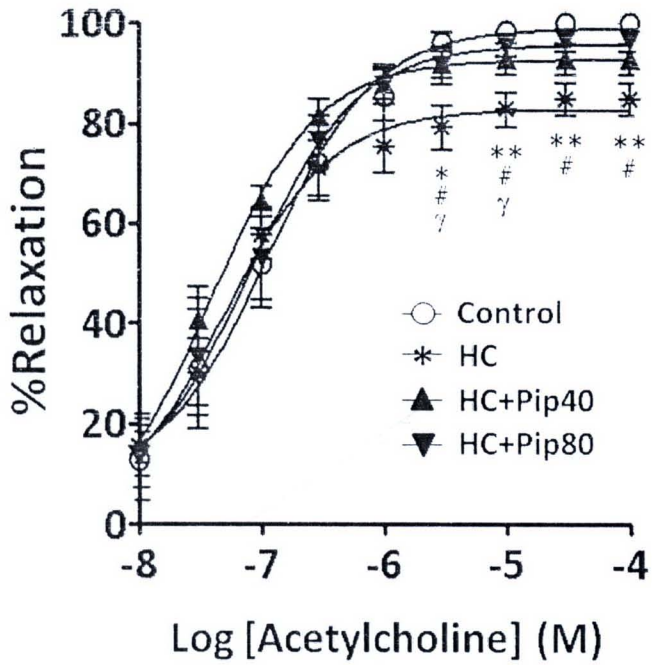

Figure 2

Concentration-response curves for acetylcholine (0.01-10μM) induced-relaxations of isolated aortae removed from the animals immediately after killing at week8. Rats were fed daily with water (control), cholesterol (HC) or cholesterol with either 40mg/kg piperine (Pip40), or 80mg/kg piperine (Pip80) as indicated. The relaxations are expressed as % reduction of tension in vessels pre-contracted with phenylephrine: 100% relaxation = vessel tension before adding phenylephrine (10μM). P-values using ANOVA are indicated $*p<0.05$, $**p<0.01$ (compared to control); $^{\#}p<0.05$ (compared to HC+Pip80); $^{\gamma}p<0.05$ (compared to HC+Pip40). All data points are mean±SEM (n=6-8).

Treatment	Serum lipid (mg/dL)					
	Total cholesterol		Triglycerides		HDL	
	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8
Control	88.6±1.5	$109.4\pm3.7^{*\gamma}$	61.1±6.9	$108.3\pm7.9^{*}$	37.3±0.7	$28.6\pm0.6^{**}$
HC	89.6±3.4	$148.3\pm8.8^{**\#}$	$76.2\pm5.7^{\#}$	$124.9\pm11.1^{*}$	35.1±1.9	$26.9\pm1.1^{*}$
HC+Pip40	88.1±2.4	$160.3\pm11.4^{**\#\#}$	$82.1\pm2.7^{\#\#}$	$82.1\pm13.5^{\gamma}$	37.3±0.8	$23.8\pm1.9^{**}$
HC+Pip80	84.5±2.2	$122.2\pm3.4^{**\Delta}$	73.8±5.2	$77.9\pm8.2^{\gamma\gamma}$	34.7±1.6	$22.3\pm1.9^{**\#}$

Table 1

Effect of piperine on serum concentrations of total cholesterol (TC), triglycerides (TG), and high density lipoprotein (HDL) measured at week 0 and week 8, expressed as mg/dL for all parameters. Rats were fed daily with water (Control), cholesterol (HC) or cholesterol with either 40mg/kg piperine (Pip40), or 80mg/kg piperine (Pip80) as indicated. P values using pair T-test (for W0 vs W8 of the same group/parameter) or ANOVA (for comparison between the 4 groups) are indicated $*p<0.01$, $**p<0.001$ (compared to week0); $^{\#}p<0.05$, $^{\#\#}p< 0.01$ (compared to control); $^{\gamma}p<0.05$, $^{\gamma\gamma}p<0.01$ (compared to HC); $^{\Delta}p<0.01$ (compared to Pip40). All data points are mean±SEM (n=6-8).