Effects of puerarin supplementation on cardiovascular disease risk factors: a randomised, double-blind, placebo-controlled, two-way crossover trial (abridged secondary publication)

MK Kwok, GM Leung *, L Xu, HF Tse, TH Lam, TH So, CM Schooling *

KEY MESSAGES

1. In healthy Chinese men in Hong Kong, short-term (12-week) supplementation with puerarin granules (90.2 mg daily) did not improve cardiovascular disease risk profile including lipid profile, blood pressure, testosterone, inflammation, coagulation, liver function, and renal function.
2. Fasting glucose was reduced after puerarin supplementation. Further research is needed to determine whether puerarin can help to improve glycaemic traits.

Hong Kong Med J 2023;29(Suppl 4):S18-21
HMRF project number: 14151121

[1] MK Kwok, [1] GM Leung, [2] L Xu, [3] HF Tse, [1] TH Lam, [4] TH So, [1,5] CM Schooling

[1] *School of Public Health, The University of Hong Kong, Hong Kong SAR, China*
[2] *School of Public Health, Sun Yat-sen University, Guangzhou, China*
[3] *Department of Medicine, School of Clinical Medicine, The University of Hong Kong, Hong Kong SAR, China*
[4] *Department of Clinical Oncology, School of Clinical Medicine, The University of Hong Kong, Hong Kong SAR, China*
[5] *City University of New York Graduate School of Public Health and Health Policy, New York, NY, United States*

* Principal applicant: gmleung@hku.hk
Corresponding author: cms1@hku.hk

Introduction

Pueraria (also known as 'Gegen' in Chinese and 'Kudzu' in Japanese), the root of *Pueraria lobata*, is a commonly used herb in Chinese medicine. According to the Pharmacopoeia of the People's Republic of China, dried pueraria is indicated for the treatment of fever, diarrhoea, diabetes, and hypertension, with a recommended daily dose of 10-15 g. Puerarin is the major bioactive constituent in pueraria. Over the past few decades, puerarin injections have been extensively used in China for the treatment of cerebrovascular ischaemia, cardiovascular disease (CVD), angina pectoris, cardiac infarction, and viral myocarditis. Puerarin capsules have been used in supplement form and are generally considered free of adverse effects. Previous *in vitro* and *in vivo* studies indicated that puerarin may have positive effects on the cardiovascular system.

Clinical trials have revealed conflicting results concerning the effects of puerarin on CVD risk factors in humans, although two studies showed beneficial effects on lipids and blood pressure.[1,2] Some trials investigating puerarin as an alternative to hormonal replacement therapy have shown a modest effect on cholesterol. Another trial indicated that puerarin supplementation improved insulin resistance.[3] However, a Cochrane review found that puerarin injection had a non-significant effect on survival or dependency in people with ischaemic stroke (relative risk=0.79, 95% confidence interval=0.45-1.36).[4] Another Cochrane review demonstrated that puerarin injection had a neutral effect on reducing episodes of acute angina (relative risk=0.95, 95% confidence interval=0.85-1.07).[5] Overall, the limited number of studies and small number of participants in each trial preclude definitive conclusions regarding the effect of puerarin on CVD risk, although the available evidence indicates possible benefits. This study assessed the effects of puerarin supplementation on CVD risk factors (lipid profile, blood pressure, and fasting glucose) and potential mediating pathways (including testosterone, inflammation, coagulation, and liver and renal function) in Chinese men.

Methods

This randomised, double-blind, placebo-controlled, two-way crossover trial included Chinese men aged 18 to 50 years who were willing to make return visits, were not currently taking any traditional Chinese medicine (including puerarin) supplementation, were not receiving hormone replacement therapy (currently or in the previous 12 months), were free of any congenital diseases or infectious diseases (eg, seasonal influenza), had no history of any chronic diseases including coronary heart disease (ischaemic heart disease), myocardial infarction (heart attack), stroke, diabetes, or cancer, and had a 10-year risk of ischaemic heart disease <10%. Participants were recruited by advertisements across various channels (eg, the university-wide bulk mailing system, in-class announcements, recruitment booths, and posters)

throughout Hong Kong. The trial was registered (reference: NCT03676296) and ethics approval was obtained before recruitment of participants.

Initial eligibility assessments were conducted on-site or via telephone. Participants were randomly allocated to one of two intervention sequences at a 1:1 ratio: puerarin then placebo or placebo then puerarin. Frontline staff, laboratory technicians, investigators, data analysts, and participants were masked to the intervention sequences for all participants.

Each participant took either a puerarin supplement or a placebo for 12 weeks; this was followed by a 4-week washout period, after which the participant was switched to the other intervention for 12 weeks. Fasting blood samples were collected at four timepoints: after randomisation, after 12 weeks of the first intervention, after 4 weeks of washout, and after 12 weeks of the second intervention). In total, 21 mL of fasting blood were collected at each sampling time point.

One sachet of puerarin granules contained a mixture of puerarin (90.2 mg) and excipients. The placebo was prepared with the same excipients but lacked puerarin. All granules were purchased from an approved Good Manufacturing Practice–certified manufacturer. The two types of granules were identical in weight and appearance.

The primary outcome was the lipid profile (levels of total cholesterol, low- and high-density lipoprotein cholesterol, and triglycerides). Secondary outcomes were CVD risk factors including blood pressure (systolic and diastolic) and fasting glucose, as well as potential mediating pathways such as testosterone, inflammation (high-sensitivity C-reactive protein), coagulation (prothrombin time), liver function (aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, total protein, and albumin), and renal function (urea, creatinine, sodium, and potassium).

An intention-to-treat analysis was performed, assuming no changes in baseline values for participants with missing follow-up values. Differences in outcomes between puerarin supplementation and placebo in participants were compared using paired t-tests or, if a period effect existed, crossover-based analysis. Statistical analyses were conducted using R software, version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The trial began on 5 September 2018 and ended on 17 April 2021. Of 277 Chinese men recruited, 217 were eligible for enrolment. Participants were randomly allocated to receive puerarin then placebo (n=112) or placebo then puerarin (n=105). Baseline characteristics of the two groups were similar (Table 1). The mean participant age was 31.4 years; 77% of the participants were born in Hong Kong, while 23% were born in Mainland China or elsewhere.

Lipid profiles were similar after puerarin or placebo supplementation; the mean difference in low-density lipoprotein cholesterol was -0.02 mmol/L (Table 2). Conversely, fasting glucose was reduced after puerarin supplementation (-0.13 mmol/L). There were no significant differences in other outcomes (ie, systolic or diastolic blood pressure, testosterone, inflammation, coagulation, liver function, or renal function).

Discussion

There was little evidence that short-term (12-week) puerarin supplementation influenced the CVD risk profile in healthy Chinese men. Lipid profiles and blood pressure values were similar, regardless of puerarin or placebo supplementation. However, fasting glucose was reduced after puerarin supplementation. Because puerarin did not influence testosterone, high-sensitivity C-reactive protein, prothrombin time, liver function, or renal function, these biomarkers presumably do not underlie mediating pathways by which puerarin is linked to health outcomes.

We found no effects of puerarin supplementation on lipid profile or blood pressure. Concerning the lipid profile, two previous trials of oral puerarin supplementation (capsules, tablets, or granules) did not indicate lipid-lowering effects; however, 3 months of puerarin supplementation at a higher dose (150 mg/day in tablet form) in addition to daily metformin led to healthier lipid profiles in women with polycystic ovary syndrome (n=30). Puerarin supplementation did not lower blood pressure in one trial, but two trials showed blood pressure reduction in patients with ischaemic stroke and/or hypertension. The mixed findings thus far do not strongly support the notion that puerarin supplementation has clear lipid- or blood pressure-lowering effects.

In this trial, we found that puerarin supplementation reduced fasting glucose. Earlier trials of puerarin supplementation did not comprehensively evaluate glycaemic status. Previous trials have shown that puerarin supplementation improves insulin resistance, as indicated by a decrease in homeostatic model assessment for insulin resistance (HOMA-IR) in patients with rheumatoid arthritis[3]; conversely, it did not improve fasting glucose in women with polycystic ovary syndrome.[2] In a rodent model of diabetic injury, puerarin supplementation enhanced glycaemic status, as indicated by improvements in insulin secretion and resistance, as well as a decrease in serum glucose; these effects were potentially mediated by the reduction of oxidative stress through anti-

TABLE 1. Baseline characteristics of participants

Baseline characteristic	Overall (n=217)*	Puerarin then placebo (n=112)*	Placebo then puerarin (n=105)*	P value
Age, y	31.4±9.2	30.8±9.2	32.0±9.2	0.32
Education				0.26
Postgraduate	100 (46.5)	51 (45.9)	49 (47.1)	
Undergraduate	102 (47.4)	56 (50.5)	46 (44.2)	
Secondary or below	13 (6.0)	4 (3.6)	9 (8.7)	
Place of birth				0.46
Hong Kong	167 (77.0)	89 (79.5)	78 (74.3)	
Mainland China or elsewhere	50 (23.0)	23 (20.5)	27 (25.7)	
Smoking status				0.13
Never-smoker	185 (88.1)	97 (89.8)	88 (86.3)	
Ex-smoker	7 (3.3)	1 (0.9)	6 (5.9)	
Current smoker	18 (8.6)	10 (9.3)	8 (7.8)	
Alcohol use				0.77
Never-drinker	47 (21.7)	23 (20.5)	24 (22.9)	
Ex-drinker	55 (25.3)	27 (24.1)	28 (26.7)	
Current drinker	115 (53.0)	62 (55.4)	53 (50.5)	
Height, cm	172.6±6.8	173.1±7.1	172.1±6.6	0.31
Body mass index, kg/m^2	23.3±3.5	23.0±3.3	23.6±3.7	0.15
Systolic blood pressure, mmHg	120.1±11.5	119.3±11.1	121.1±12.0	0.25
Diastolic blood pressure, mmHg	75.9±10.1	74.7±9.9	77.2±10.2	0.06
Waist circumference, cm	85.4±10.9	84.4±11.5	86.5±10.2	0.16
Hip circumference, cm	97.3±7.7	96.8±8.0	97.9±7.4	0.27
Total body fat, %	21.8±17.4	20.9±12.9	22.8±21.2	0.43
Total muscle mass, %	53.3±7.5	53.2±8.3	53.4±6.3	0.88
Triglycerides, mmol/L	1.07±0.93	1.05±0.96	1.10±0.90	0.68
Total cholesterol, mmol/L	4.71±0.92	4.70±0.98	4.71±0.86	0.90
High-density lipoprotein cholesterol, mmol/L	1.34±0.34	1.34±0.33	1.34±0.35	0.98
Low-density lipoprotein cholesterol, mmol/L	2.90±0.81	2.91±0.85	2.89±0.77	0.89
Fasting glucose, mmol/L	5.17±0.59	5.13±0.61	5.20±0.58	0.36
Testosterone, nmol/L	20.43±7.85	20.66±7.15	20.18±8.56	0.65
C-reactive protein, mg/L	1.20±1.96	1.24±2.14	1.17±1.76	0.79
Prothrombin time, seconds	11.66±1.11	11.67±0.98	11.66±1.23	0.97
Aspartate aminotransferase, U/L	23.24±29.16	24.30±39.09	22.10±11.53	0.58
Alanine aminotransferase, U/L	34.07±23.42	31.55±18.81	36.76±27.34	0.10
Alkaline phosphatase, U/L	67.70±18.76	67.20±17.81	68.24±19.80	0.68
Gamma-glutamyl transferase, U/L	33.94±26.98	31.61±19.48	36.42±33.08	0.19
Total bilirubin, μmol/L	10.33±4.48	10.51±4.82	10.14±4.10	0.55
Total protein, g/L	75.54±4.09	75.36±4.38	75.74±3.75	0.49
Albumin, g/L	43.55±2.45	43.54±2.36	43.55±2.56	0.98
Urea, mmol/L	5.27±1.21	5.14±1.07	5.42±1.33	0.09
Creatinine, μmol/L	83.41±12.05	83.35±10.55	83.47±13.51	0.94
Sodium, mmol/L	139.01±1.61	139.04±1.60	138.97±1.63	0.74
Potassium, mmol/L	4.58±0.39	4.55±0.37	4.60±0.41	0.40

* Data are presented as mean ± standard deviation or No. (%) of participants

inflammatory processes, such as tumour necrosis factor-related pathways. Further investigations of various glycaemic traits (eg, fasting glucose, fasting insulin, glycated haemoglobin, insulin resistance, and beta-cell function) in humans are needed to clarify the effects of puerarin on glycaemic control.

Notably, puerarin supplementation had no effects on testosterone, inflammation, coagulation, renal function, or liver function in healthy Chinese men. Puerarin binds to oestrogen receptors with potentially weak antagonistic properties; it is not known to directly influence androgenic activities, although indirect modulation may occur through increased levels of serum sex hormone binding globulin.[2] Puerarin supplementation counteracted the anticoagulation effect of warfarin in rodents, but its ability to exert a similar effect in humans has not yet been examined. Previous studies in rodents have also shown that puerarin is well-tolerated by the kidneys and liver.

There were a few limitations in this trial. First, it was conducted during the COVID-19 pandemic, which delayed or disrupted recruitment and in-person clinical follow-up sessions. We recruited additional participants to allow for a high number of dropouts. Second, some outcomes may have been influenced by period effects. To adjust for these effects, we conducted crossover-based analyses of the relevant outcomes. Third, participants were highly educated; however, this presumably did not affect the internal validity of the findings because the randomisation approach minimises confounding and selection bias at the time of recruitment. Finally, the trial only included men; the findings require validation in other populations to confirm their generalisability.

TABLE 2. Treatment effects or crossover effects (if period effect is significant) of puerarin supplementation

Outcome	Mean difference (95% confidence interval)	P value
Triglycerides, mmol/L	-0.008 (-0.12 to 0.11)	0.897
Total cholesterol, mmol/L	-0.03 (-0.11 to 0.06)	0.515
High-density lipoprotein cholesterol, mmol/L	0.003 (-0.02 to 0.03)	0.833
Low-density lipoprotein cholesterol, mmol/L	-0.02 (-0.09 to 0.06)	0.648
Systolic blood pressure, mmHg	-0.35 (-1.91 to 1.20)	0.652
Diastolic blood pressure, mmHg	-0.08 (-1.53 to 1.37)	0.912
Fasting glucose, mmol/L	-0.13 (-0.25 to -0.008)	0.036
Testosterone, nmol/L	0.52 (-0.37 to 1.42)	0.250
C-reactive protein, mg/L	0.33 (-0.72 to 1.37)	0.541
Prothrombin time, seconds	0.04 (-0.13 to 0.21)	0.604
Aspartate aminotransferase, U/L	-1.99 (-5.73 to 1.74)	0.295
Alanine aminotransferase, U/L	-0.64 (-3.35 to 2.08)	0.644
Alkaline phosphatase, U/L	-0.35 (-1.82 to 1.12)	0.639
Gamma-glutamyl transferase, U/L	1.25 (-2.17 to 4.66)	0.473
Total bilirubin, μmol/L	-0.09 (-0.92 to 0.75)	0.840
Total protein, g/L	-0.62 (-1.26 to 0.03)	0.060
Albumin, g/L	-0.22 (-0.63 to 0.20)	0.303
Urea, mmol/L	-0.01 (-0.22 to 0.19)	0.912
Creatinine, nmol/L	0.14 (-1.15 to 1.43)	0.832
Sodium, mmol/L	-0.27 (-0.63 to 0.09)	0.140
Potassium, mmol/L	-0.001 (-0.07 to 0.07)	0.980
Height, cm	6.87 (-2.55 to 16.28)	0.152
Body mass index, kg/m^2	0.76 (-0.63 to 2.16)	0.283
Waist circumference, cm	-0.26 (-1.13 to 0.61)	0.556
Hip circumference, cm	0.07 (-1.06 to 1.19)	0.903
Total muscle mass, %	-2.77 (-7.57 to 2.02)	0.256
Total body fat, %	-0.05 (-2.32 to 2.22)	0.965

Conclusion

In healthy Chinese men in Hong Kong, short-term (12-week) puerarin supplementation (90.2 mg daily) did not improve CVD risk profile including the lipid profile, blood pressure, testosterone, inflammation, coagulation, liver function, and renal function. Further research is needed to determine whether puerarin can help to improve glycaemic traits such as fasting glucose.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#14151121). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Kwok MK, Leung GM, Xu L, Tse HF, Lam TH, Schooling CM. Effect of puerarin supplementation on cardiovascular disease risk factors: a randomized, double-blind, placebo-controlled, 2-way crossover trial. Biomed Pharmacother 2022;153:113472.

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