Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Meta-Analysis

The antiosteoporosis effect of icariin in ovariectomized rats: a systematic review and meta-analysis

Yu Liu[1#], Haojiang Zuo[1#], Xuewei Liu[1], Jingyuan Xiong[2*], Xiaofang Pei[2*]

[1] Department of Public Health Laboratory Sciences, West China School of Public Health, Sichuan University, Chengdu, 610041 China
[2] Research Center for Public Health and Preventive Medicine, West China School of Public Health, Sichuan University, Chengdu, 610041 China

Correspondence to: jzx0004@tigermail.auburn.edu; xxpeiscu@163.com

[#] These two authors made equal contributions

Received August 22, 2017; **Accepted** November 25, 2017; **Published** November 30, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.11.22

Abstract: *Herba Epimedii* (Yinyanghuo or Horny Goat Weed) is a traditional Chinese medicine widely used in treating osteoporosis. As the major active component, icariin is intensively investigated in the prevention and treatment of osteoporosis in ovariectomized rats. However, misleading conclusions can be generated in animal studies with various experimental designs. Therefore, a systematic review and meta-analysis was performed to evaluate the efficacy of icariin against osteoporosis in ovariectomized rats. PubMed, EMBASE and Chinese National Knowledge Infrastructure databases were searched to identify studies of icariin in ovariectomized rats. Two independent authors selected and reviewed the publications. Data were pooled using a DerSimonian and Laird random-effects model. The results demonstrated that ovariectomized rats treated with icariin had significantly higher bone mineral density (femur and lumbar spine) and lower bone turnover markers (serum alkaline phosphatase and osteocalcin) compared with the ovariectomized control group. For bone histomorphometric parameters, the percentages of trabecular area and trabecular thickness were significantly higher while the trabecular separation was significantly lower in the ovariectomized rats treated with icariin. Based on these results, the presnet study suggested that icariin might possess substantial antiosteoporosis effect in ovariectomized rats. Safety studies and large randomized clinical trials are needed to further support possible clinical applications of icariin in postmenopausal women with osteoporosis.

Key words: Icariin; Osteoporosis; Ovariectomized rats; Systematic review; Meta-analysis.

Introduction

Osteoporosis is a metabolic and systematic skeleton disease characterized by low bone mass and microarchitectural deterioration of bone tissue, resulting in increased bone fragility and risk of fracture (1, 2). It is a critical health problem throughout the world, and prevalently reported in postmenopausal women whose estrogen levels decrease continuously (3) . Although the hormone replacement therapy (HRT) was efficacious in preventing bone loss and reducing the incidence of fractures (4-7), the Women's Health Initiative Study and the Million Women Study indicated that HRT was liable to the increasing risks of breast cancer, stroke, thrombosis and cardiovascular diseases in postmenopausal women (8-10). In addition, serious adverse effects were prominent for other therapeutic agents including calcitonin, bisphosphonates and selective estrogen receptor modulators (SERMs) (11-15). Therefore, effective alternatives with fewer side effects are in demand for the prevention and treatment of osteoporosis in postmenopausal women. Recently, plants derived products attracted wide attentions in nutritional and pharmacological studies due to their natural origin, mild toxicity and relatively low cost.

Herba Epimedii (Yinyanghuo or Horny Goat Weed) is a flowering plant majorly grown in China and other Asian countries. In traditional Chinese medicine (TCM), the use of *Herba Epimedii* in treating osteoporosis has a long standing history (16). However, direct clinical application of *Herba Epimedii* is limited due to the uncertainty and complications of constituents in the herbal formulae. Icariin ($C_{33}H_{40}O_{15}$, molecular weight: 676.67), a prenylated flavonol glycoside, is the major active component of *Herba Epimedii* (17, 18). The effects of icariin on the prevention and treatment of osteoporosis in ovariectomized rats were investigated in a number of studies. However, these studies varied from each other in many aspects including the design of study, which could lead to the inconsistency of the results and conclusions. In addition, the results of these animal studies were often obtained from relatively small sample sizes, which could increase the risk of insufficient statistical power in concluding statistical differences. Thus, any study relying on a single non-decisive animal experiment could be misleading in the future clinical researches and applications, which in turn could result in exhausting limited resources.

Systematic review and meta-analysis are considered to be a valuable tool in assisting the evaluation of animal experiments in clinical researches. They can provide important insights into the validity of animal studies, improve the precision of estimated effects, and support further generalization to human clinical trials (19). Therefore, systematic review and meta-analysis of studies reporting the efficacy of icariin in ovariectomized rats may contribute greatly to the interpretation and aggregation of data, and ascertain the potential

benefit of icariin in treating postmenopausal osteoporosis. The previous meta-analysis by Xu et al. reported that icariin had an effect on improving bone mineral density (BMD) in ovariectomized rats (20). Except for the reduced BMD, in postmenopausal osteoporosis, microarchitectural deterioration of trabecular bone is another feature in the early stage of postmenopausal osteoporosis. Thus, bone histomorphometry analysis could help us understand the trabecular bone loss. Besides, lack of estrogen could enhance bone metabolism, while serum pharmacology is one of the important methods to study bone metabolism. Accordingly, this prospective systematic review and meta-analysis were conducted in conjunction, comparing serum bone metabolism markers and bone histomorphometric parameters other than BMD between icariin treatment group and control group in ovariectomized rats, with the aim to provide a better understanding of the efficacy of icariin against osteoporosis in ovariectomized rats.

Materials and Methods

Literature search and inclusion criteria

Two independent researchers performed all the literature searches for relevant studies of icariin in ovariectomized rats of osteoporosis in PubMed, EMBASE and Chinese National Knowledge Infrastructure (CNKI) by December 2016. Search keywords included icariin, osteoporosis, antiosteoporosis and bone. No language or other limitations were imposed on the search process. Reference lists from the resulting publications and reviews were checked manually to identify additional relevant publications.

To prevent bias, inclusion criteria were pre-specified as followings: (1) published literature after peer review; (2) the icariin treatment and control groups were comprised of ovariectomized rats; (3) administered icariin as an intervention; (4) at least one of the following outcomes was reported: bone mineral density (femur bone mineral density (F-BMD), lumbar spine bone mineral density (L-BMD)), bone turnover markers (serum alkaline phosphatase (S-ALP), serum osteocalcin (S-OC)), bone histomorphometric parameters (percentage of trabecular area (% Tb.Ar), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb. Sp)).

Reviews and duplications were excluded. If the data were included in multiple publications, either the study with the earliest publication date or the study with the most complete set of information was included.

Two independent authors (Yu Liu and Haojiang Zuo) reviewed and assessed the inclusion and exclusion of studies carefully for this systematic review and meta-analysis. Any minor discrepancies were resolved though discussion or by consensus.

Methodological quality appraisal

Quality of each included study was assessed using the following criteria (21, 22): (1) publication after peer review; (2) random allocation to treatment or control groups; (3) calculation of sample size; (4) blinded assessment of outcome; (5) compliance with animal welfare regulations; (6) husbandry conditions (e.g. light/dark cycle, quality of water, access to food and water, type of food, temperature, breeding program, and environment enrichment). Each study was given a quality score out of a maximum total of 6 points.

Data extraction

Two authors (Yu Liu and Haojiang Zuo) independently extracted the following data from each eligible study: (1) the surname of the first author, the year of publication and rat species; (2) treatment information including dosage, time of administration, route of drug delivery and duration of treatment; (3) number of rats in icariin treatment group and control group, mean value and standard deviation (SD) of the outcomes. The time of bilateral ovariectomy was set to zero and the time of administration was expressed accordingly. When the outcomes were determined at different times, only the final measurement was included. If the data used for the meta-analysis were missing or only expressed graphically, corresponding authors were first contacted to request further information. If no response was received, the studies with the missing data were omitted from the analysis altogether, and the studies with only graphic data were analyzed using digital ruler software. All data were entered into a standardized Excel (Microsoft Corp) file and were checked by a third author (Xiaofang Pei). If there were any disagreements, consensus was reached through discussions.

Statistical analyses

The heterogeneity across the included studies was tested using Cochran's Q-statistic (23) and I^2 statistic (24). A P value below 0.1 was considered statistically significant for the heterogeneity. An I^2 value less than 25% was considered homogeneous. An I^2 value between 25% and 50% was considered low heterogeneity. An I^2 value between 50% and 75% was considered moderate heterogeneity. An I^2 value above 75% was considered high heterogeneity. Data were pooled using a DerSimonian and Laird random-effects model owning to the high heterogeneity in the systematic review of animal experiments (25). Since the outcomes were determined in various ways and their mean values varied greatly for different studies, standardized mean differences (SMDs) and 95% confidence intervals (95% CIs) were calculated (26). Moreover, in order to explore the impact of study characteristics on the estimates of combined effect size, pre-specified subgroup analyses were conducted according to the followings: rat species, publication status, reported study quality score, time of administration, duration of treatment and icariin dosage. In order to ensure the reliability of results, a sensitivity analysis was performed. Potential publication bias was assessed by the visual inspection of the funnel plot and asymmetry test. The subgroup analyses, sensitivity analyses and assessment of publication bias were carried out only for F-BMD due to the limited numbers of other outcomes in the studies.

The significance level was set at $P < 0.05$. Significant differences between subgroups were assessed by partitioning heterogeneity with the χ^2 distribution and n-1 degrees of freedom (n equals to the number of subgroups). All analyses were performed using Stata version 12.0 (StataCorp, College Station, TX).

Results

Eligible studies and study quality

A total of 258 studies were identified by the initial database search. 75 duplicated studies were omitted and 166 studies were excluded after reviewing the titles and abstracts. The remaining 17 full-text articles were scrutinized for more detailed evaluations. Three articles were excluded because they were essentially the same study with multiple publications. Finally, 14 studies (27-40) with 354 ovariectomized rats (225 in the icariin treatment group and 129 in the control group) met the inclusion criteria and were included in this systematic review and meta-analysis. The literature search and study selection process was shown in Figure 1. The summary of study design characteristics was listed in Table 1. Out of the 14 studies, 11 studies received a quality score of 3 or greater. The lowest quality score was 2 (31,33,35), and the highest score was 4 (26-30,39). No study mentioned the calculation of sample size and none used a blinding method during outcome assessment. All studies reported the random allocation of ovariectomized rats to groups but none of them provided the precise method of randomization (Table 2).

Overall efficacy

F-BMD was determined in ten studies (27-31, 35-38, 40). A total of 16 comparisons provided numerical data and the pooled results showed that the icariin treatment group significantly demonstrated higher F-BMD than the ovariectomized control group (SMD=1.497, 95% CI=1.097 to 1.897, *P*=0.000) (Figure 2a). When the F-BMD values calculated by the digital ruler software from the study by Cheng et al. were included in the meta-analysis (38), the pooled results revealed the similar tendency (SMD=1.505, 95% CI=1.133 to 1.877, *P*=0.000) (Figure 2b).

L-BMD was determined in seven studies (30, 31, 34, 36-39). According to the pooled results from the numerical data in ten comparisons, the icariin treatment group showed a significantly higher L-BMD compared with the ovariectomized control group (SMD=2.330, 95% CI=1.519 to 3.140, *P*=0.000) (Figure 3a). When the L-BMD values calculated by the digital ruler software from the study by Cheng et al. were included in the meta-analysis (38), the analysis showed the similar results

Table 1. Summary of characteristics of included studies.

Study			Number[b]		Intervention				
author	year	Rat species[a]	C	T	dosage	Time of administration[c]	Route of drug delivery	Duration of treatment	Outcome[d]
Nian(27)	2009	SD	10	10 10 10	5mg/kg/d 25mg/kg/d 125mg/kg/d	unclear	orally	12 weeks	S-ALP, S-OC, F-BMD, Tb.Th, Tb.Sp
Qin(28)	2008	SD	10	10 10	25mg/kg/d 125mg/kg/d	1 day	unclear	12 weeks	F-BMD, % Tb.Ar, Tb.Th, Tb.N, Tb.Sp
Yang(29)	2014	SD	10	10	20mg/kg/d	12 weeks	intragastric administration	12 weeks	S-ALP, S-OC, F-BMD, % Tb.Ar, Tb.Th, Tb.N, Tb.Sp
Liu(30)	2012	SD	7	8	12mg/kg/d	7 days	oral gavage	3 months	F-BMD, L-BMD
Li(31)	2014	SD	9	9	125mg/kg/d	7 days	orally administration	12 weeks	F-BMD, L-BMD
Bian(32)	2011	SD	3	3	20mg/kg/d	1 day	intragastric administration	12 weeks	Tb.Th, Tb.N, Tb.Sp
Qian(33)	2009	SD	9	9 9 9	40mg/kg/d 80mg/kg/d 160mg/kg/d	1 week	intragastric administration	12 weeks	S-OC
Wu(34)	2011	SD	12	12 12 12	25mg/kg/d 50mg/kg/d 100mg/kg/d	1 week	intragastric administration	4 months	L-BMD
Bao(35)	2005	Wistar	6	6 6 6	75mg/kg/d 150mg/kg/d 225mg/kg/d	2 weeks	intragastric administration	8 weeks	F-BMD
Song(36)	2012	Wistar	20	20	300mg/kg/d	30 days	intragastric administration	90 days	S-ALP, F-BMD, L-BMD
Bai(37)	2010	SD	6	6 6 6	75mg/kg/d 150mg/kg/d 225mg/kg/d	3 days	intragastric administration	8 weeks	S-ALP, F-BMD, L-BMD
Cheng(38)	2014	SD	12	12	25mg/kg/d	1 week	intragastric administration	3 months	S-OC, F-BMD, L-BMD, Tb.Th, Tb.N, Tb.Sp,
Liu(39)	2012	SD	6	6	4mg/kg/d	5 days	intragastric administration	13 weeks	L-BMD
Xue(40)	2012	SD	8	8	20mg/kg/d	12 weeks	intragastric administration	12 weeks	S-ALP, F-BMD, % Tb.Ar, Tb.Th, Tb.N, Tb.Sp

[a] C: the ovariectomized control group; T: the icariin treatment group

[b] The time of bilateral ovariectomy was set to zero and the time of administration expressed accordingly

Table 2. Risk of bias summary.

study		Publication after peer review	Random allocation to treatment or control groups	Calculation of sample size	Blinded assessment of outcome	Compliance with animal welfare regulations	Husbandry conditions	Score
author	year							
Nian(27)	2009	√	√			√	√	4
Qin(28)	2008	√	√			√	√	4
Yang(29)	2014	√	√			√	√	4
Liu(30)	2012	√	√			√	√	4
Li(31)	2014	√	√			√	√	4
Bian(32)	2011	√	√					2
Xue(40)	2012	√	√			√	√	4
Qian(33)	2009	√	√				√	3
Wu(34)	2011	√	√					2
Bao(35)	2005	√	√				√	3
Song(36)	2012	√	√					2
Bai(37)	2010	√	√				√	3
Cheng(38)	2014	√	√				√	3
Liu(39)	2012	√	√				√	3

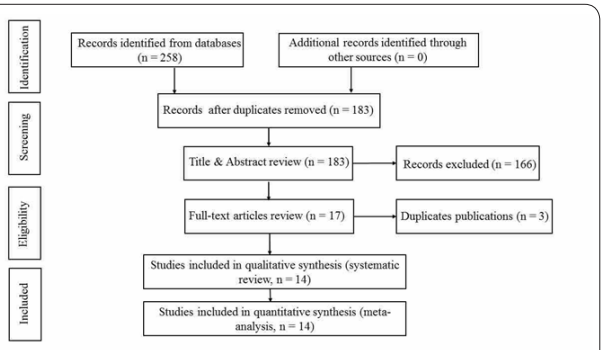

Figure 1. Flow chart of literature search and study selection.

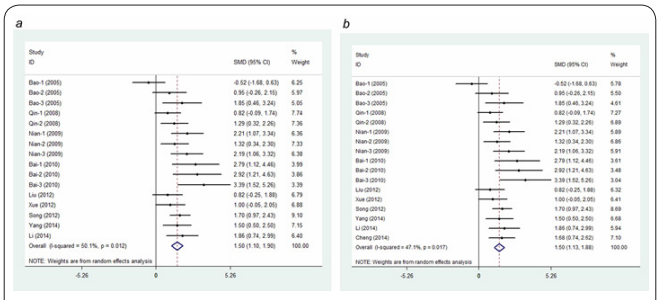

Figure 2. Forest plot of the difference in F-BMD between the icariin treatment group and the ovariectomized control group: (a) pooled results without graphical data (b) pooled results with graphical data.

(SMD=2.201, 95% CI=1.475 to 2.927, *P*= 0.000) (Figure 3b).

% Tb.Ar was determined in three studies (28, 29, 40). Analysis by a SMD method with the random-effects model revealed that the icariin treatment group had significantly higher % Tb.Ar than the ovariectomized control group (SMD=1.612, 95% CI=0.666 to 2.557, *P*=0.001) (Figure 4).

Tb.Th was determined in six studies (27-29, 32, 38, 40). The analysis with nine comparisons showed that the Tb.Th was significantly higher in the icariin treatment group when compared with the ovariectomized control group (SMD=3.961, 95% CI=2.389 to 5.533, *P*=0.000) (Figure 5).

Tb.Sp was determined in six studies (27-29, 32, 38, 40). The Tb.Sp was significantly lower in the icariin treatment group than that in the ovariectomized control group (SMD=-3.014, 95% CI =-4.337 to -1.691, *P*=0.000) (Figure 6).

Tb.N was determined in five studies (28, 29, 32, 38, 40). The Tb.N was higher in the icariin treatment group than that in the ovariectomized control group. However, the significance level was not reached (SMD=0.909, 95% CI=-0.060 to 1.878, *P*=0.066) (forest plot not shown).

S-ALP levels were determined in five studies (27, 29, 36, 37, 40) and one studies expressed the values of S-ALP levels in the graphic form (29). Digital ruler software was adopted to calculate these graphic data. For all the studies with or without graphic data of S-ALP levels, the pooled results showed that S-ALP levels were significantly lower in the icariin treatment

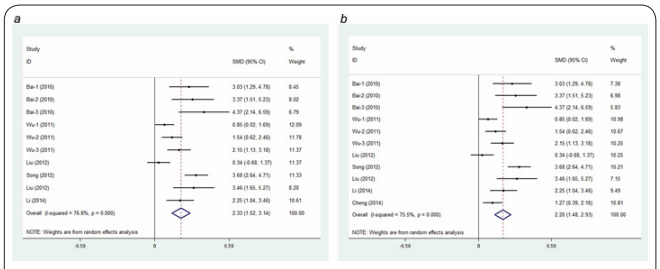

Figure 3. Forest plot of the difference in L-BMD between the icariin treatment group and the ovariectomized control group: (a) pooled results without graphical data (b) pooled results with graphical data.

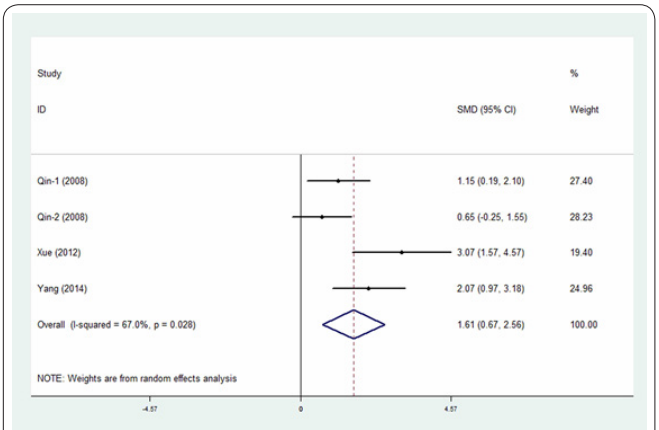

Figure 4. Forest plot of the difference in % Tb.Ar between the icariin treatment group and the ovariectomized control group.

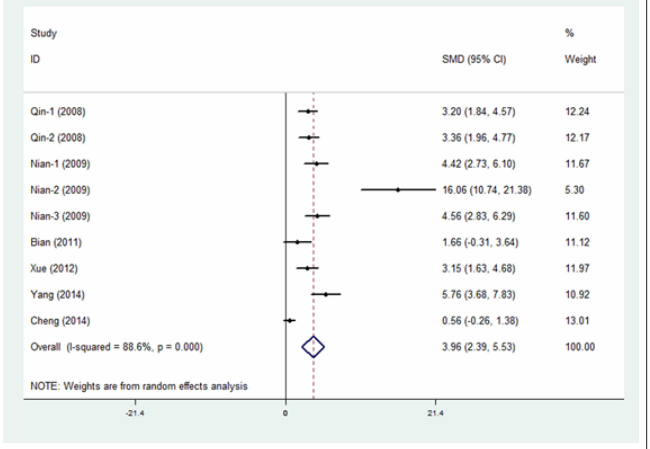

Figure 5. Forest plot of the difference in Tb.Th between the icariin treatment group and the ovariectomized control group.

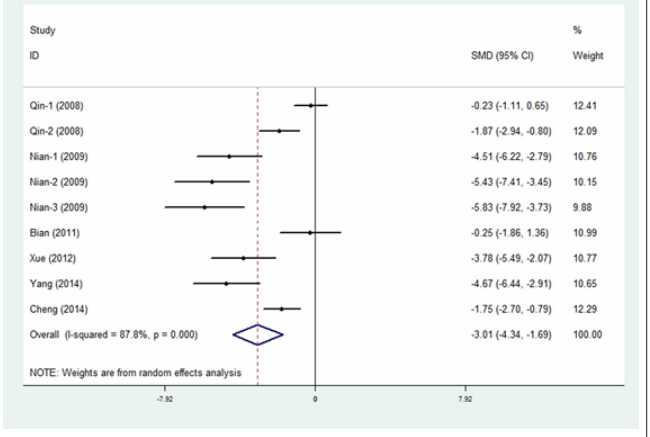

Figure 6. Forest plot of the difference in Tb.Sp between the icariin treatment group and the ovariectomized control group.

group when compared with the ovariectomized control group (for studies without graphic data, SMD=-3.319, 95% CI=-5.187 to -1.451, *P*=0.000; for studies with graphic data, SMD=-2.976, 95% CI =-4.522 to -1.430, *P*=0.000) (Figure 7).

S-OC levels were significantly lower in the icariin treatment group than those in the ovariectomized group (for studies without graphic data, SMD=-4.081, 95% CI=-6.282 to -1.881, *P*=0.000; for studies with graphic data from the study by Yang et al. (29), SMD =-3.542, 95% CI=-5.439 to -1.646, *P*=0.000) (Figure 8).

Impact of study characteristics

No efficacy difference between SD rats and Wistar rats was observed (χ^2=1.95, df=1, *P*=0.163, Figure 9a). There seemed to be a trend for the magnitude of efficacy to be greater with shorter intervals between surgery and treatment. However, no significant differences in the time of administration were determined (χ^2=3.55, df=3, *P*=0.314, Figure 9b). In addition, the duration of treatment showed no significant difference on the effect of icariin (χ^2=0.02, df=1, *P*=0.889, Figure 9c). For icariin dosage, there was a trend for magnitude of efficacy to be greater with the larger dosage. However, this trend did not reach the prespecified significance threshold (χ^2=2.83, df=3, *P*=0.419, Figure 9d).

Sensitivity analysis and publication bias

Sensitivity analysis was carried out to assess the influence of the publication status of the studies. After omitting the studies not formally published (37, 38), the pooled result showed that the icariin treatment group still demonstrated significantly higher F-BMD than the ovariectomized control group (SMD=1.306, 95% CI=0.935 to 1.678, *P*=0.000). Besides, considering that Bao et al. (34) and Liu et al. (38) did not specifically reported the use of DXA in BMD measurement, sensitivity analysis omitting these two studies were also performed. Results showed that F-BMD and L-BMD were still significantly higher in the icariin treatment than those in the ovariectomized control group (F-BMD:

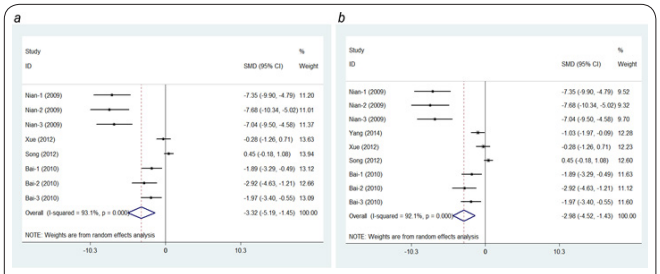

Figure 7. Forest plot of the difference in S-ALP between the icariin treatment group and the ovariectomized control group: (a) pooled results without graphical data (b) pooled results with graphical data.

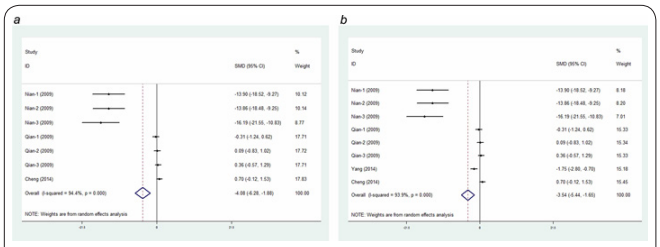

Figure 8. Forest plot of the difference in S-OC between the icariin treatment group and the ovariectomized control group: (a) pooled results without graphical data (b) pooled results with graphical data.

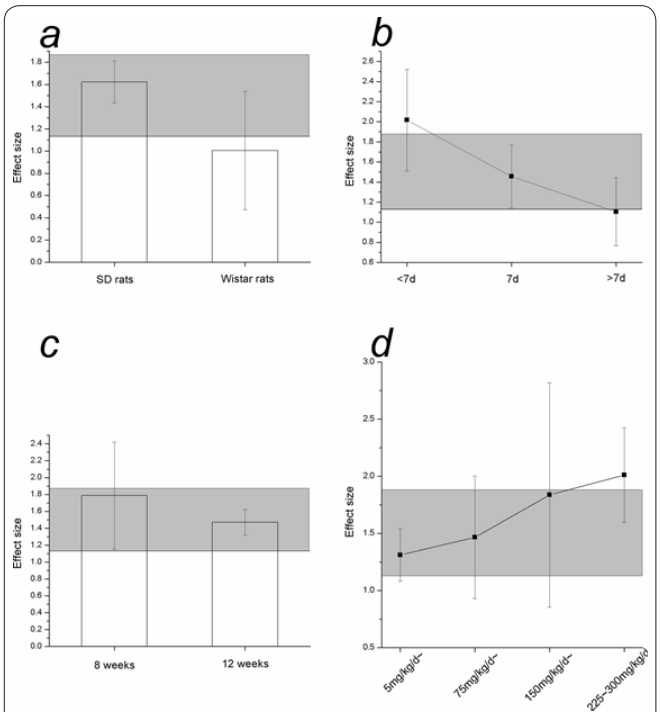

Figure 9. Subgroup analysis: point estimates and 95% CIs of the effect size by (a) rat species; (b) time of administration; (c) duration of treatment: (d) icariin dosage. The 95% CI for the global estimate is shown as a grey band.

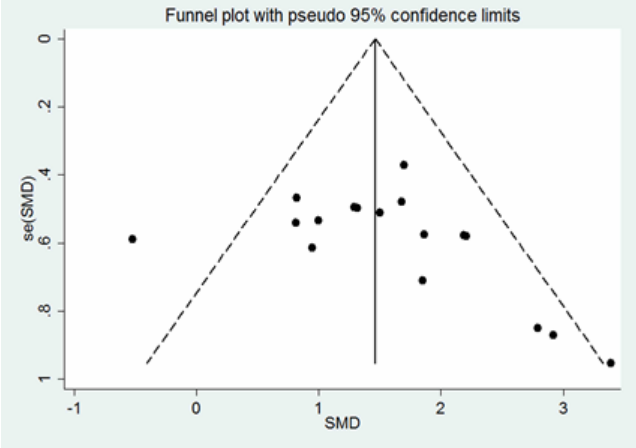

Figure 10. Funnel plot for the effect size of icariin treatment on F-BMD.

SMD=1.626, 95% CI=1.263 to 1.990, *P*=0.000; L-BMD: SMD=2.228, 95% CI=1.384 to 3.072, *P*=0.000), suggesting that results of the current meta-analysis were robust and reliable.

The funnel plot was slightly asymmetrical for the effect of icariin on F-BMD through visual inspection, indicating a mild publication bias existed (Egger's test, *P*=0.117) (Figure 10).

Discussion

Herba Epimedii has long been used in traditional Chinese antiosteoporosis formulae. Being the major active constituent of *Herba Epimedii*, the efficacy of icariin against osteoporosis was investigated in multiple animal studies. In order to draw clinical relevant conclusions on the efficacy of icariin against osteoporosis, a prospective systematic review and meta-analysis on the antiosteoporosis effect of icariin in ovariectomized rats was adopted.

Lumbar spine and femur are clinically recommended sites for BMD measurement, while most researchers measured BMD of these two sites in ovariectomized rats treated with icariin. According to the present study, bone mineral density (BMD) at femur and lumbar spine were significantly higher in the icariin treatment group compared with the ovariectomized control group. Since the bilateral ovariectomy could significantly reduce estrogen secretion and subsequently decrease bone density decreases (41), these findings suggested that the treatment of icariin was able to inhibit the reduction of BMD in ovariectomized rats. In addition, the values of Tb.Ar and Tb.Th were significantly higher, while the value of Tb.Sp was significantly lower in the icariin treatment group than those in the ovariectomized control group. Previous studies demonstrated that lack of estrogen could lead to increase in the number of bone-resorbing osteoclasts and decrease in the number of bone-forming osteoblasts (42, 43). When bone resorption exceeded the bone formation, the imbalanced bone remodeling process occurred. Ultimately, the enhanced bone remodeling led to excessive loss of bone mass in trabecular than cortical bone (44). The results of the current meta-analysis indicated that icariin was able to inhibit the mass loss of trabecular bone. Moreover, significant estrogen deficiency in postmenopausal women and ovariectomized rats results in high bone turnover. High bone turnover in the early stage can be indicated by enhanced levels of two osteoblast activity markers, S-ALP and S-OC (45-47). Thus, in order to provide a better understanding of the efficacy of icariin against osteoporosis in ovariectomized rats, data of S-ALP and S-OC have been pooled and analyzed in this meta-analysis. In this study, significantly lower levels of S-ALP and S-OC were observed in the ovariectomized rats treated with icariin compared with the ovariectomized control group, indicating that icariin inhibited the bone remodeling in ovariectomized rats. Recent researches reported that icariin not only was able to stimulate the proliferation and differentiation of osteoblasts (48, 49), but also suppressed osteoclastic differentiation (50) through up-regulating bone metabolism related mRNA expressions of multiple molecules, including bone morphogenic protein-2 (BMP-2), Smad4, osteoprotegerin (OPG), Cbfa1/Runx2 and OPG/RANKL ratio (51-56). Together with the reported results from this meta-analysis, it is evident that icariin can inhibit BMD decrease, mass loss of trabecular bone and bone remodeling, supporting the antiosteoporosis effect in the ovariectomized rats.

The ovariectomized rat is a well established model for researches on postmenopausal osteoporosis supported by the U.S. Food and Drug Administration (U.S. FDA) and the World Health Organization (WHO) (42, 57) due to the prominent characteristics resembling human postmenopausal osteoporosis (58). A systematic review and meta-analysis of pre-clinical animal experiments could increase the precision of estimated treatment effects (59), and may assist in the selection of the most promising treatment strategies for future clinical trials (60). Based on the key findings in this study, icariin is highly likely to be an effective candidate for treating osteoporosis in postmenopausal women. A previous study suggested that icariin was a more potent antiosteoporosis agent than genistein, a major soybean isoflavone speculated to be an osteoblast stimulator and osteoclast inhibitor (61). Further more, icariin was demonstrated to be free of uterotrophic effects (51, 53), which strongly increased the possibilities of applying icariin in the prevention and treatment of postmenopausal osteoporosis. However, while all included studies in this meta-analysis did not report any side effects or death during the treatment with icariin in ovariectomized rats, the pharmacokinetics and safety of icariin are yet to be explored. In studies analyzing the effect of icariin on osteoporosis, ovariectomized rats were the preferred animal models that mimic the high bone turnover osteoporosis. Future studies should be focused on determining the effect of icariin on the low bone turnover osteoporosis models, to provide more details of pharmacological effects of icariin.

According to the study quality appraisal results, the overall study quality was modest. Eleven studies received a quality score of 3 or greater. The highest score was 4 and the lowest score was 2. No study adopted a blinding method, rendering a risk of reporting positive effect (62). Accordingly, the true efficacy of icariin might be overestimated in this study.

This meta-analysis had some limitations. A mild publication bias existed in our meta-analysis. However, the publication bias is inevitable in any meta-analysis

due to delayed publication bias (63). Besides, there was significant heterogeneity between studies due to variation in study quality and experimental designs (64). Thus, the random-effects model was adopted to minimize the risk of providing erroneous estimates.

In conclusion, the findings in this study suggested that icariin might provide appreciable preventive effects against osteoporosis in ovariectomized rats. Due to the lack of safety data, further studies investigating the safety issues of icariin for postmenopausal osteoporosis are highly recommended. In addition, large randomized clinical trials evaluating the efficacy of icariin in postmenopausal women with osteoporosis are also required.

Acknowledgments

The authors would like to thank Tao Zhou, Tianli Zheng and Jiayi Chen for their helpful discussions regarding this manuscript. This work was supported by Sichuan University Talent Introduction Start-up Fund (Jingyuan Xiong).

Conflict of interest

The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest. 2005;115:3318-3325.
2. Osteoporosis prevention, diagnosis, and therapy. JAMA. 2001;285:785-795.
3. Hansen MA, Overgaard K, Riis BJ, Christiansen C. Potential risk factors for development of postmenopausal osteoporosis--examined over a 12-year period. Osteoporos Int. 1991;1:95-102.
4. Lindsay R, Hart DM, Aitken JM, MacDonald EB, Anderson JB, Clarke AC. Long-term prevention of postmenopausal osteoporosis by oestrogen. Evidence for an increased bone mass after delayed onset of oestrogen treatment. Lancet. 1976;1:1038-1041.
5. Fitzpatrick LA. Estrogen therapy for postmenopausal osteoporosis. Arq Bras Endocrinol Metabol. 2006;50:705-719.
6. Lindsay R, Hart DM, Clark DM. The minimum effective dose of estrogen for prevention of postmenopausal bone loss. Obstet Gynecol. 1984;63:759-763.
7. Blank RD, Bockman RS. A review of clinical trials of therapies for osteoporosis using fracture as an end point. J Clin Densitom. 1999;2:435-452.
8. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288:321-333.
9. Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2003;362:419-427.
10. Gambacciani M, Ciaponi M, Genazzani AR. The HRT misuse and osteoporosis epidemic: a possible future scenario. Climacteric. 2007;10:273-275.
11. Papaioannou A, Kennedy CC, Dolovich L, Lau E, Adachi JD. Patient adherence to osteoporosis medications: problems, consequences and management strategies. Drugs Aging. 2007;24:37-55.
12. Watts NB, Diab DL. Long-term use of bisphosphonates in osteoporosis. J Clin Endocrinol Metab. 2010;95:1555-1565.
13. O'Regan RM, Gradishar WJ. Selective estrogen-receptor modulators in 2001. Oncology (Williston Park). 2001;15:1177-1185, 1189-1190; discussion 1190-1174.
14. Body JJ. Calcitonin for the long-term prevention and treatment of postmenopausal osteoporosis. Bone. 2002;30:75-79.
15. Watts NB. Bisphosphonate treatment of osteoporosis. Clin Geriatr Med. 2003;19:395-414.
16. Zhai YK, Guo X, Pan YL, Niu YB, Li CR, Wu XL, et al. A systematic review of the efficacy and pharmacological profile of Herba Epimedii in osteoporosis therapy. Pharmazie. 2013;68:713-722.
17. Feng D, Gao ZY. Determination of the content of icariin in Liuweixianshenzhi tablets with HPLC. Chin Pharm 2001; 12: 42-43.
18. Gao G, Liao M, Feng Y. (Determination of flavonoids and quality evaluation of Chinese traditional drug "puhuang"). Yao Xue Xue Bao. 1998;33:300-303.
19. Pound P, Roberts I. The need for systematic reviews of animal studies. Chin J Evid Based Med 2005; 5: 3-5.
20. Xu JH, Yao M, Ye J, Wang GD, Wang J, Cui XJ, et al. Bone mass improved effect of icariin for postmenopausal osteoporosis in ovariectomy-induced rats: a meta-analysis and systematic review. Menopause. 2016;23:1152-1157.
21. Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke. 2004;35:1203-1208.
22. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol. 2010;160:1577-1579.
23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21:1539-1558.
24. Zintzaras E, Ioannidis JP. Heterogeneity testing in meta-analysis of genome searches. Genet Epidemiol. 2005;28:123-137.
25. Jove M, Pamplona R, Prat J, Arola L, Portero-Otin M. Atherosclerosis prevention by nutritional factors: a meta-analysis in small animal models. Nutr Metab Cardiovasc Dis. 2013;23:84-93.
26. Wen J, Li YP. The selection of a summary statistic for use in meta-analysis. Chin J Evid Based Med 2007; 7: 606-613.
27. Nian H, Ma MH, Nian SS, Xu LL. Antiosteoporotic activity of icariin in ovariectomized rats. Phytomedicine. 2009;16:320-326.
28. Qin L, Han T, Zhang Q, Cao D, Nian H, Rahman K, et al. Antiosteoporotic chemical constituents from Er-Xian Decoction, a traditional Chinese herbal formula. J Ethnopharmacol. 2008;118:271-279.
29. Yang L, Yu Z, Qu H, Li M. Comparative effects of hispidulin, genistein, and icariin with estrogen on bone tissue in ovariectomized rats. Cell Biochem Biophys. 2014;70:485-490.
30. Liu M, Zhong C, He RX, Chen LF. Icariin associated with exercise therapy is an effective treatment for postmenopausal osteoporosis. Chin Med J (Engl). 2012;125:1784-1789.
31. Li GW, Xu Z, Chang SX, Nian H, Wang XY, Qin LD. Icariin prevents ovariectomy-induced bone loss and lowers marrow adipogenesis. Menopause. 2014;21:1007-1016.
32. Bian Q, Liu SF, HUang JH, Yang Z, Ning Y, Zhao YJ, Wang YJ, Shen ZY. Regulation and control of three active components of TCM effective in reinforcing kidney on ovariectomy-induced osteoporosis rats's bone marrow stem cells revealed by gene microarray. Chin J TCM 2011; 26: 889-893.
33. Qian GF, Yang H, Zhang XZ. Effect of icariin on the expression of Cbfa1in the bone of ovariectomized rats. Chin J Osteoporosis & Bone Miner Res 2009; 2: 104-109.
34. Wu MS, Zhao SZ, Ren LZ, Wang R, Bai X. Effects of icariin on expression of the estrogen receptor beta mRNA of bone and different hypothalamic nuclei in ovariectomy rats. Chin Pharm Bulletin 2011; 27: 29-33.
35. Bao JR, Yang JW, Li SF, Zhao W, Zhang QL, Yan YQ. Effects of icarrin on ovariectomized rats. J Hygene Res 2005; 34: 191-193.
36. Song Y, Li SH, He ZJ, Zhang YW, Zhou MW. Effect of icariin on osteoporosis in ovariectomized female rats. J Chin PLA Postgard

Med Sch 2012; 33: 400-403.
37. Bai J. Effects of icariin on ovariectomized osteoporotic rats (Dissertation). Lanzhou (China): Lanzhou University of Technology 2010.
38. Cheng K. Comparative research of icariin and genistein of pharmacological activity in antiosteoporosis (Dissertation). Lanzhou (China): Lanzhou University of Technology 2014.
39. Liu SJ, Mo XM, Li JP, Yang J, Zhang XJ. Effecs of icariin and possible mechanisms in ovariectomized rats. Chin J Basic Med in TCM 2012; 3: 271-275.
40. Xue L, Wang Y, Jiang Y, Han T, Nie Y, Zhao L, et al. Comparative effects of er-xian decoction, epimedium herbs, and icariin with estrogen on bone and reproductive tissue in ovariectomized rats. Evid Based Complement Alternat Med. 2012;2012:241416.
41. Aswar UM, Mohan V, Bodhankar SL. Antiosteoporotic activity of phytoestrogen-rich fraction separated from ethanol extract of aerial parts of Cissus quadrangularis in ovariectomized rats. Indian J Pharmacol. 2012;44:345-350.
42. Kalu DN. The ovariectomized rat model of postmenopausal bone loss. Bone Miner. 1991;15:175-191.
43. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med. 1995;332:305-311.
44. Lerner UH. Bone remodeling in post-menopausal osteoporosis. J Dent Res. 2006;85:584-595.
45. Mukaiyama K, Kamimura M, Uchiyama S, Ikegami S, Nakamura Y, Kato H. Elevation of serum alkaline phosphatase (ALP) level in postmenopausal women is caused by high bone turnover. Aging Clin Exp Res. 2014.
46. Yogesh HS, Chandrashekhar VM, Katti HR, Ganapaty S, Raghavendra HL, Gowda GK, et al. Anti-osteoporotic activity of aqueous-methanol extract of Berberis aristata in ovariectomized rats. J Ethnopharmacol. 2011;134:334-338.
47. Hertrampf T, Schleipen B, Offermanns C, Velders M, Laudenbach U, Diel P. Comparison of the bone protective effects of an isoflavone-rich diet with dietary and subcutaneous administrations of genistein in ovariectomized rats. Toxicol Lett. 2009;184:198-203.
48. Qian WQ, Yin H, Sun HT. Influence of different concentrationsof icariin on osteoblast metabolism of rats. Chin Med Herald 2011; 8: 23-25.
49. Guo HL, Zhao YF, Wang X, Xu Y, Zhan HS. Experimental study on the mechanism of icariin improving human osteoblasts proliferation and the expression of OPG protein. Chin J Orthop Trauma 2011; 24: 585-588.
50. Huang J, Yuan L, Wang X, Zhang TL, Wang K. Icaritin and its glycosides enhance osteoblastic, but suppress osteoclastic, differentiation and activity in vitro. Life Sci. 2007;81:832-840.
51. Cao H, Ke Y, Zhang Y, Zhang CJ, Qian W, Zhang GL. Icariin stimulates MC3T3-E1 cell proliferation and differentiation through up-regulation of bone morphogenetic protein-2. Int J Mol Med. 2012;29:435-439.
52. Zhao J, Ohba S, Komiyama Y, Shinkai M, Chung UI, Nagamune T. Icariin: a potential osteoinductive compound for bone tissue engineering. Tissue engineering Part A. 2010;16:233-243.
53. Mok SK, Chen WF, Lai WP, Leung PC, Wang XL, Yao XS, et al. Icariin protects against bone loss induced by oestrogen deficiency and activates oestrogen receptor-dependent osteoblastic functions in UMR 106 cells. Br J Pharmacol. 2010;159:939-949.
54. Zheng D, Peng S, Yang SH, Shao ZW, Yang C, Feng Y, et al. The beneficial effect of Icariin on bone is diminished in osteoprotegerin-deficient mice. Bone. 2012;51:85-92.
55. Hsieh TP, Sheu SY, Sun JS, Chen MH, Liu MH. Icariin isolated from Epimedium pubescens regulates osteoblasts anabolism through BMP-2, SMAD4, and Cbfa1 expression. Phytomedicine. 2010;17:414-423.
56. Liang W, Lin M, Li X, Li C, Gao B, Gan H, et al. Icariin promotes bone formation via the BMP-2/Smad4 signal transduction pathway in the hFOB 1.19 human osteoblastic cell line. Int J Mol Med. 2012;30:889-895.
57. Bagi C, van der Meulen M, Brommage R, Rosen D, Sommer A. The effect of systemically administered rhIGF-I/IGFBP-3 complex on cortical bone strength and structure in ovariectomized rats. Bone. 1995;16:559-565.
58. Jee WS, Yao W. Overview: animal models of osteopenia and osteoporosis. J Musculoskelet Neuronal Interact. 2001;1:193-207.
59. Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I. Where is the evidence that animal research benefits humans? BMJ. 2004;328:514-517.
60. van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al. Can animal models of disease reliably inform human studies? PLoS Med. 2010;7:e1000245.
61. Ma HP, Ming LG, Ge BF, Zhai YK, Song P, Xian CJ, et al. Icariin is more potent than genistein in promoting osteoblast differentiation and mineralization in vitro. J Cell Biochem. 2011;112:916-923.
62. Bebarta V, Luyten D, Heard K. Emergency medicine animal research: does use of randomization and blinding affect the results? Acad Emerg Med. 2003;10:684-687.
63. Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ. 2011;343:d4002.
64. Macleod MR, O'Collins T, Horky LL, Howells DW, Donnan GA. Systematic review and metaanalysis of the efficacy of FK506 in experimental stroke. J Cereb Blood Flow Metab. 2005;25:713-721.