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

Yu Liu1, Haojiang Zuo1, Xuewei Liu1, Jingyuan Xiong2*, Xiaofang Pei2*

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: jxx0004@tigermail.auburn.edu; xxpeiscu@163.com

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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 present 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) is 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 (C34H34O15, 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...
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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 ovarietomized 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 ovarietomized rats, with the aim to provide a better understanding of the efficacy of icariin against osteoporosis in ovarietomized rats.

Materials and Methods

Literature search and inclusion criteria

Two independent researchers performed all the literature searches for relevant studies of icariin in ovarietomized 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 ovarietomized 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 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 ovarietomy 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 F statistic (24). A P value below 0.1 was considered statistically significant for the heterogeneity. An F value less than 25% was considered homogeneous. An F value between 25% and 50% was considered low heterogeneity. An F value between 50% and 75% was considered moderate heterogeneity. An F 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 χ² 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.

<table>
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<tr>
<th>Study</th>
<th>Number</th>
<th>Intervention</th>
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<th>Route of drug delivery</th>
<th>Duration of treatment</th>
<th>Outcome</th>
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<tr>
<td>author</td>
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<td>Rat species*</td>
<td>C (mg/kg/d)</td>
<td>T (mg/kg/d)</td>
<td>C (days)</td>
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*C: the ovariectomized control group; T: the icariin treatment group

The time of bilateral ovariectomy was set to zero and the time of administration expressed accordingly.
Table 2. Risk of bias summary.

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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.

Figure 3. Forest plot of the difference in % Tb.Ar between the icariin treatment group and the ovariectomized control group.

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

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 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=0.660 to 1.878, \( P=0.066 \) ) (forest plot not shown).

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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 (\(\chi^2=1.95, \text{df}=1, P=0.163\), 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 (\(\chi^2=3.55, \text{df}=3, P=0.314\), Figure 9b). In addition, the duration of treatment showed no significant difference on the effect of icariin (\(\chi^2=0.02, \text{df}=1, P=0.889\), 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 (\(\chi^2=2.83, \text{df}=3, P=0.419\), 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 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.

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.
The efficacy of icariin on the mass loss of trabecular bone. Moreover, significant meta-analysis indicated that icariin was able to inhibit deling led to excessive loss of bone mass in trabecu process occurred. Ultimately, the enhanced bone removal of osteoblasts (42, 43). When bone resorption exceeded osteoclasts and decrease in the number of bone-forming S-Ap and S-OC have been pooled and analyzed in this meta-analysis. In this study, significantly lower levels of S-Ap 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 differentiatution (50) through up-regulating bone metabolism related mRNA expressions of multiple molecules, including bone morphogenetic 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 antioestoporosis 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). Furthermore, 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. High bone turnover in the early stage can be indicated by enhanced levels of two osteoblast activity markers, S-Ap 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-Ap and S-OC have been pooled and analyzed in this meta-analysis. In this study, significantly lower levels of S-Ap 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.

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).

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

**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 ova-
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.

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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.

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