Systematic review of preclinical evidence reveals the anti-inflammatory potential of Icariin based on meta-analysis and machine learning

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Abstract

Purpose: The aim of this study was to construct a reference system for preclinical evidence and drug superiority characterization of the anti-inflammatory effects of icariin glycosides and their derivatives through meta-analysis combined with machine learning, and to excavate the biological mechanisms behind them. Methods: The data were obtained from databases of PubMed, Embase, Cochrane Library, and Web of Science. STATA software was used for meta-analysis of indicators, and subgroup analysis was conducted based on animal species, gender, type of disease, drug dosage, and course to obtain more particulars. Furthermore, model construction were performed using R software to explore influential features on drug efficacy. In addition, the pharmacological mechanisms of icariin and its derivatives in anti-inflammation were summarized based on a comprehensive understanding of relevant literature. Results: After searching and screeningThe results showed that icariin and its derivatives significantly inhibit inflammation indicators such as TNF-α and IL-1β. Besides, machine learning with TNF-α as the output variable showed that icariin and its derivatives had stronger anti-inflammatory effects when the type of disease was respiratory, urological, neurological, and digestive, and when the dose and duration of Icariin were greater than 27.52 mg/kg/day and 31.22 days, respectively. Conclusion: Icariin and its derivatives demonstrate strong anti-inflammatory effects, particularly for respiratory, urinary, neurological, and digestive disorders. When given at doses of 27.52 mg/kg/day or more, with treatment lasting 31.22 days or beyond, these compounds hold significant potential as drugs for inflammation inhibition across multiple dis

1 Introduction

As a paramount fundamental pathological mechanism, inflammation exerts a pivotal influence on the pathogenesis of numerous diseases, and its timely attenuation is imperative to prevent potential deleterious consequences on the organism.(Kong et al., 2022). The mechanism of the inflammatory response is intricate, involving a range of cytokines like TNF-α and IL-1β, as well as significant signaling pathways like NF-ϰB and P38 MAPK. Inhibiting these inflammatory processes presents a promising avenue for effectively treating associated diseases.

Icariin (PubChem CID: 5318997), as a flavonol glycoside compound purified from the traditional Chinese medicine Epimedium, which is also known as "Yin Yang Huo", has variety of bioactive components(M. Wang, Gao, Li, & Wu, 2020). It has been studied extensively and being considered as a potential therapeutic medicine for many diseases, and demonstrated to exert anti-inflammation(G. Wang et al., 2022), anti-oxidative(J. Jin et al., 2019), anti-apoptotic effect(L. Hu et al., 2019) and anti-tumor(Gao, Wang, & Gao, 2022). Increasing evidence has shown that icariin plays a key role in prevention and treatment of many diseases by mitigating inflammatory response. Therefore, it is of great significance to study the effect of

icariin on anti-inflammation and to explore the underlying mechanism. The research roadmap is as follow (Figure1).

Accumulating evidence from a large number experiments is essential to draw definitive conclusions about potential therapies. However, current*in vivo* studies often report inconsistent results and excessive animal testing raises ethical concerns(Ioannidis et al., 2014; Locker, 2004). Statistical synthesis of data from multiple in vivo studies will help address these issues by providing a more precise and objective assessment with fewer experiments. In particular, systematic reviews of data have the potential to accelerate drug translation. In recent years, this approach has been applied to several interventions, such as melatonin for myocardial ischemia-reperfusion injury and quercetin for liver fibrosis(X. Guo et al., 2022; Mao, Lin, Xiao, Huang, & Chen, 2020). Careful consideration of drug dosage and regimen is crucial, as they can impact animal model results. Advancements in machine learning now allow tapping into drug efficacy for different disease categories, guiding preclinical and early clinical study design, including ideal dose intervals and durations(Greener, Kandathil, Moffat, & Jones, 2022). In conclusion, systematic evaluation combined with machine learning enhances the effectiveness and translational value of preclinical studies. It also provides valuable reference for subsequent animal experiments, improving experimental efficacy.

2 METHOD

2.1 Meta analysis

2.1.1 Search strategy

A comprehensive search was performed on the major databases, namely PubMed, Embase, Cochrane Library, and Web of Science, covering all publications since their establishment up to March 2023, without any publication restrictions. To ensure a comprehensive search, the appropriate search term "treatment combined with disease" was selected for identifying relevant studies on icariin's efficacy in inhibiting inflammation. Keywords related to icariin, Epimedium, Yin Yang Huo, inflammation, and inflammatory disease were searched in the databases (Supplementary Form 1).

2.1.2 Inclusion criteria

The included studies met the following conditions: (1) Inflammatory disease animal models are induced through methods such as physical ligation or chemical induction. (2) Animal model species, gender, age, weight, and sample size were not restricted. (3) The treatment group received only icariin or its derivatives, while the control group received either vehicle treatment or no treatment. Main inflammatory markers were Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β). Secondary inflammatory markers were Interleukin-6 (IL-6), Interferon gamma (IFN-γ), Transforming Growth Factor beta-1 (TGF-β1), Inhibitor kappa B α (IkB-α), Nuclear factor NF-kappa-B (NF-ϰB) p65 subunit, and Nucleotide-binding oligomerization domain (NLRP3). The anti-inflammatory/transcriptional regulators were Peroxisome Proliferator-Activated Receptor alpha (PPARα), Peroxisome Proliferator-Activated Receptor gamma (PPARγ) Antioxidative stress indicators were Superoxide Dismutase (SOD) and Malondialdehyde (MDA). The apoptosis regulators are B-cell lymphoma-2 (BCL-2) and Bcl-2 Assaciated X, protein (Bax).

2.1.3 Exclusion citeria

The following types of documents are excluded: (1) Comments, reviews, conference reports, in vitro studies, and clinical studies. (2) Diseases that are not in inflammatory or non-inflammatory pathological phase. (3) Literature that has not been published. (4) Duplicate publications.

2.1.4 Data extraction

Two researchers independently extracted the following information from the literature: (1) The author's name and publication year. (2) Details of the animal model used in the study, including the type, gender, and sample size. (3) The method used to induce inflammation in the model. (4) The method used to induce anesthesia. (5) Details of the treatment drugs used in the intervention group, including the name, dose and timing of treatment. (6) Details of the treatment drugs used in the model group, including the name, dose

and timing of treatment. For included drug doses, the highest dose was recorded. If the data were presented in graphical form, the original author was contacted to obtain the data. If the original data could not be obtained, digital ruler software was used to measure the data from the image.

2.1.5 Risk of bias assessment

To evaluate the quality of literature on the use of icariin for treating inflammatory diseases, a risk of bias Assessment was conducted using the SYRCLE list proposed by Hooijmans.et al (Hooijmans et al., 2014). The assessment consisted of 10 items, including: (1) Sequence generation; (2) Baseline characteristics; (3) Allocation concealment; (4) Random housing; (5) Blinding of performance bias; (6) Random outcome assessment; (7) Blinding detection bias; (8) Incomplete outcome data; (9) Selective outcome reporting; (10) Other sources of bias. Each study was given a total score of ten points, with each item worth one point. The Risk of Bias Assessment was performed independently by two researchers, and in case of any discrepancies, the results were reviewed by a third researcher for arbitration.

2.1.6 Statistical analysis

In this study, since the outcome indicators were continuous variables, the effect sizes were combined using the standardized mean difference (SMD) with 95% confidence intervals and the random effects model was used to pool effect size. A significant difference between the experimental and control groups was indicated by a P-value < 0.05. To evaluate heterogeneity, the I-square (I^2) was used, and values of I^2 [?] 50% indicated that the included studies were homogeneous. If $I^2 >$ 50%, it indicated that the included studies existed heterogeneity, and subgroup analyses were conducted to explore the sources of heterogeneity. The combined effect sizes were then combined using random effects models.

2.2 Machine Learning

2.2.1 Research object and variable selection

The data source used in this study is the literature included in the Meta-analysis, and its inclusion and exclusion criteria are the same as those of the Meta-analysis. However, it is important to note that data from different studies with different sample sizes may introduce potential weighting factors leading to interfere with results in machine learning. We collected samples for all doses and durations in each literature. The relevant sample data of each document is interpolated with the average value of the corresponding index of the document. The sample data consisted of independent variables such as dose and treatment time. The dependent variable is a continuous variable, and we use TNF-α improvement rate ((Mean of control group-Mean of experimental group)/Mean of control group) as an index to evaluate the effectiveness of inflammation treatment.

2.2.2 Data processing

The data set contains a total of 260 samples, and the relevant data is highly discrete, while data standardization (convert the mean of the sample to 0 and the mean to 1) has the advantages of making the data distribution more concentrated, eliminating the impact of dimensional differences between data, and increasing the interpretability of the machine learning model(De Livera et al., 2015). Therefore, data normalization was performed on our dataset to improve the data structure.

2.2.3 Construction and evaluation of predictive models

In this study, Stacking Ensemble Learning was used to predict the level of TNF-α. The 4 basic models included in the stacking ensemble learning are as follows: Random Forest (RF), Extreme Gradient Boosting (XGBoost) model, Least Absolute Shrinkage and Selection Operator (Lasso), Single Hidden Layer Neural Network (SHLNN). We use data standardization to optimize the data, and use v-fold for 5-fold cross-validation (v =5), and each fold performs 10 random divisions ($break$ =10) as a resampling method. Finally, set up the model and use the grid search method to optimize the hyperparameters of the corresponding model, and use cross-validation to evaluate the model performance of the corresponding parameters. Afterwards, the constructed model is added to the Stacking Ensemble Model as a meta-feature, and lasso regression is

used to fit the meta-model features to obtain a better model, and then the model is used to predict and evaluate the training set and the test set respectively. At the same time, use Root Mean Squared Error (RMSE), R-squared (R^2) and Mean Absolute Error (MAE) as evaluation indicators to evaluate the Stacking Ensemble Model.

2.2.4 Model interpretation

Machine learning models can be difficult to interpret, but the SHapley Additive explanations (SHAP) method, which is based on game theory and was proposed by Lundberg, et al.(Lundberg & Lee, 2017), can help to overcome this challenge by providing accurate explanations of the model's output. The SHAP method ranks the importance of each feature in the input data based on its SHAP value, where a higher SHAP value indicates a greater positive impact on the machine learning model, while a lower SHAP value indicates a smaller impact or even a negative impact.

2.3 Analysis software and version

The software used in this study includes: STATA (version 15.0), R (version 4.2.3) and Python (version 3.11.3).

3 RESULTS

During the initial literature search, 942 articles were found. After removing duplicates, 550 articles remained. Next, 201 articles were selected for full-text review after reviewing the titles and abstracts. Finally, 19 articles were included in the analysis after a thorough reading of the full text (Y. Deng et al., 2016; Fu et al., 2018; J. Guo et al., 2010; Y. Hu et al., 2016; H. Li et al., 2018; L. Li et al., 2014; P. Ma, Zhang, Su, Qiu, & Wu, 2015; Shao et al., 2015; Su et al., 2018; Tao et al., 2013; Wei et al., 2015; Wu et al., 2011; Xie et al., 2018; Xiong et al., 2016; C. Q. Xu et al., 2010; F. Zhang et al., 2015; L. Zhang, Wang, Li, Zhang, & Hao, 2017; X. Zhang et al., 2018; J. Zhou et al., 2011)(**Figure 2**).

3.1 Characteristics of the included studies

The 19 included literatures are all in English, and a total of 370 animals were included, including 180 animals in the experimental group and 190 animals in the control group **(Figure 3)** . Animal species include Sprague-Dawley rats(SD rats)(Y. Deng et al., 2016; J. Guo et al., 2010; Xiong et al., 2016; F. Zhang et al., 2015; L. Zhang et al., 2017), C57BL mice(H. Li et al., 2018; Shao et al., 2015; Tao et al., 2013; Wu et al., 2011; Xie et al., 2018; C. Q. Xu et al., 2010), BALB/c mice(L. Li et al., 2014; P. Ma et al., 2015; Wei et al., 2015; J. Zhou et al., 2011), Wistar rats(Fu et al., 2018; Y. Hu et al., 2016), MRL/lpr mice(Su et al., 2018) and Minipigs(X. Zhang et al., 2018). Inflammatory diseases studied include local inflammation(L. Li et al., 2014; Wu et al., 2011; X. Zhang et al., 2018), circulatory system inflammation(Fu et al., 2018; Y. Hu et al., 2016), urinary system inflammation(P. Ma et al., 2015; Su et al., 2018; Xie et al., 2018; L. Zhang et al., 2017), nervous system inflammation(Y. Deng et al., 2016; J. Guo et al., 2010; H. Li et al., 2018; Xiong et al., 2016), respiratory system inflammation(Wei et al., 2015; C. Q. Xu et al., 2010), Digestive system inflammation(Tao et al., 2013; F. Zhang et al., 2015), Motion system inflammation(Shao et al., 2015) and tumor-associated inflammation(J. Zhou et al., 2011) (Table 1).

According to the inclusion and exclusion criteria, all animal models were models of inflammation-related diseases. The experimental group received monotherapy with icariin or its derivatives, and the control group received only normal saline or distilled water. In order to evaluate the effect of icariin and its derivatives on the treatment of inflammatory diseases, 12 literatures reported the level of TNF-α(Fu et al., 2018; J. Guo et al., 2010; Y. Hu et al., 2016; H. Li et al., 2018; L. Li et al., 2014; Shao et al., 2015; Su et al., 2018; Tao et al., 2013; Wu et al., 2011; Xie et al., 2018; C. Q. Xu et al., 2010; F. Zhang et al., 2015), 10 literatures reported the level of IL-1β(Y. Deng et al., 2016; Fu et al., 2018; J. Guo et al., 2010; H. Li et al., 2018; Shao et al., 2015; Su et al., 2018; Xie et al., 2018; Xiong et al., 2016; L. Zhang et al., 2017; X. Zhang et al., 2018), and 6 literatures reported the level of IL-6(Fu et al., 2018; Y. Hu et al., 2016; Shao et al., 2015; Wei et al., 2015; Xie et al., 2018; F. Zhang et al., 2015), 5 literatures reported the level of TGF-β1(Y. Deng et al., 2016; Fu et al., 2018; Wei et al., 2015; Xiong et al., 2016; L. Zhang et al., 2017), 5 literatures reported the level

of SOD(Y. Hu et al., 2016; H. Li et al., 2018; P. Ma et al., 2015; Xie et al., 2018; F. Zhang et al., 2015), 4 literatures reported the level of MDA(Y. Hu et al., 2016; H. Li et al., 2018; P. Ma et al., 2015; Xie et al., 2018), 5 literatures reported the level of IκBα(Y. Deng et al., 2016; Fu et al., 2018; L. Li et al., 2014; Xiong et al., 2016; L. Zhang et al., 2017), 4 literatures reported the level of Bcl-2 Level(H. Li et al., 2018; P. Ma et al., 2015; Xie et al., 2018; F. Zhang et al., 2015), 3 literatures reported the level of IFN-γ(Tao et al., 2013; X. Zhang et al., 2018; J. Zhou et al., 2011), 2 literatures reported the level of Bax-2(H. Li et al., 2018; Xie et al., 2018), 2 literatures reported the level of Caspase-1(Su et al., 2018; L. Zhang et al., 2017), 2 literatures reported the level of PPARα(Y. Deng et al., 2016; Xiong et al., 2016), and 2 literatures reported the level of PPARγ(Y. Deng et al., 2016; Xiong et al., 2016), 2 literatures reported the level of NLRP3(Su et al., 2018; L. Zhang et al., 2017), and 2 literatures reported the level of p65 NF-κB(Y. Deng et al., 2016; L. Zhang et al., 2017).

3.2 Study quality

Among the 19 articles included in the study, all articles describe methods for randomizing animals into experimental and control groups. 6 articles described possible prognostic features and animal factors, 16 articles described methods of concealing the order of allocation, all studies described random allocation of animal rooms, and 16 articles reported blinding of trial participants, 12 articles described random selection of animals for outcome assessment, 15 articles blinded outcome assessment, and 8 articles reported the information of experimental attrition and sample exclusion, and 10 articles reported the completeness of the data, and no other sources of bias and selective reporting were found in these 19 articles(Figure 4).

3.2 Effectiveness and mechanism of meta-analysis

3.2.1 Main indicators of inflammation

TNΦ-α

12 studies included showed that icariin and its derivatives significantly decreased the level of TNF-α [n = 238, *SMD*=-6.326, *95%CI* (-7.860, -4.791), p = 0.000] (**Figurer 5A**). Furthermore, given the significant heterogeneity among studies (I^2 = 83.4%, p = 0.000), subgroup analyzes were performed based on dose, duration of treatment, type of disease, animal species and animal sex. Among them, the heterogeneity of the "0-5 days" subgroup categorized by treatment duration and the "Local inflammation" and "Nervous system" subgroups categorized by disease type improved significantly, indicating that treatment duration and disease type are important sources of heterogeneity. Heterogeneity was significantly improved in the "0-5 days" subgroup based on treatment duration and the "Local inflammation" and "Nervous system" subgroups based on disease type, suggesting that treatment duration and disease type are important sources of heterogeneity in this index **(Table 2)** .

IΛ-1β

Icariin and its derivatives significantly reduced the level of IL-1β in the 10 studies included [n = 204, SMD =-5.698, 95%CI (-7.307, -4.089), p = 0.000] (**Figure 5B**). Given the significant heterogeneity observed across studies (I^2 = 84.1%, p = 0.000), subgroup analysis was performed to explore the sources of heterogeneity. In the subgroup analysis by species, the heterogeneity of the "mice" group was significantly improved. In addition, the heterogeneity of the "0-5 days" subgroup categorized by treatment duration was also significantly improved. This suggests that species and treatment duration are important sources of heterogeneity in the meta-analysis of IL-1β **(Table 3)** .

3.2.2 Secondary indicators of inflammation

Icariin and its derivatives showed good therapeutic effects on the relevant secondary inflammatory indices, specific information is shown in the table **(Table 4)** . Among them, icariin and its derivatives can effectively inhibit the levels of IL-6 (p = 0.000), IFN-γ (p = 0.031) and TGF-β1 (p = 0.000), and at the same time significantly increase the levels of IkB-α (p = 0.000), NF-κB p65 (p = 0.000) and NLRP3 (p = 0.000), thereby achieving anti-inflammatory effects.

3.2.3 Anti-inflammatory/transcriptional regulators

Icariin also had an ameliorative effect on anti-inflammatory/transcriptional regulators **(Table 4)** , significantly increasing PPARα levels ($p = 0.000$), and it is worth noting that the low heterogeneity of this indicator has a positive effect on the accuracy of the results, see table for more details.

3.2.4 Anti-oxidative stress indicators

The efficacy of Icariin and its derivatives in anti-oxidative stress indicators is also excellent **(Table 4)** , which can not only significantly up-regulate the SOD level ($p = 0.000$) under low heterogeneity ($I^2 = 0.0\%$, $p = 0.047$), but also can inhibit the MDA activity ($p = 0.000$), so as to achieve the effect of anti-oxidative stress, and then inhibit the process of inflammation, details are shown in table.

3.2.5 Apoptosis regulators

Icariin and its derivatives have also shown significant efficacy in the regulation of Apoptosis regulators **(Table 4)** . It significantly up-regulated BCL-2 ($p = 0.000$) while also exhibiting a potent inhibitory effect on caspase-1 ($p = 0.000$). Reassuringly, the meta-analysis of the efficacy of icariin on caspase-1 also demonstrated low heterogeneity ($I^2 = 7.9\%$, $p = 0.297$).

3.2.6 Publication bias

The results of the publication bias detection based on the main indicators of the meta-analysis are as follows. The result of Egger's test for TNF-α was t = -4.59 (Figure 6A), and its absolute value was greater than 0.05, indicating that there was no publication bias. The result of the egger's test for IL-1β was t= - 4.21(Figure 6B), and the absolute value of the t value was greater than 0.05, so it was considered that there was no publication bias.

3.3 Machine learning

3.3.1 Sample characteristics and correlation analysis

The study included a total of 260 samples. The dose range of icariin and derivatives was 3-300mg/kg/day, and the treatment period was 0.25-90 days. The time-dose-efficacy distribution is shown in the figure (Figure 7). Meanwhile, Kendall analysis was used for correlation analysis of categorical variables (Figure 8A), and Pearson analysis was used for correlation analysis of continuous variables (Figure 8B). The analysis results show that the absolute value of the correlation between each feature is less than 0.35, suggesting that the risk of collinearity is low.

3.3.2 Model Development and Evaluation

In this study, four machine learning algorithms were used to establish the meta-model, and the features of the four meta-models were fitted by using the Stacking algorithm. After hyperparameter optimization, the RMSE values of the obtained four meta-models are all lower than 0.32, and the prediction ability of the models is good. This study uses four machine learning algorithms to establish the basic model, and uses the Stacking algorithm to fit the characteristics of these four basic models. After optimization of hyperparameters, the RMSE values of the four models were all lower than 0.43, and the prediction ability of the models was good (Figure 9A). These models are then fitted using the stacking model and regularized. The results show that when the*penalty parameter* is 0.0173, the *mixture* value is 1, that is, the meta-model is completely used for prediction under this*penalty parameter* value, and the corresponding *RMSE* is 0.208, and R^2 is 0.954, indicating that the model prediction performance is good (Figure 9B). At the same time, the*RMSE* values of the meta-model in the training set and test set are 0.193 and 0.289, the R^2 is 0.962 and 0,928, and the *MAE* is 0.068 and 0.116, respectively, and the model performance is good (Figure 9C, Figure 9D).

3.3.3 Model interpretation

Construct the S HAP value variable importance map (Figure 9A) of the prediction model, which includes a total of 5 features, sorted according to their influence on the level of TNF-α, the higher the absolute value of SHAP value, the greater the effect on TNF-α powerful. The results showed that the characteristics of "Type of disease", "Animal species" and "Dose" had a higher contribution to the improvement of TNF-α level (Figure 10). In the constructed SHAP value summary graph, the higher the SHAP value corresponding to a feature, the greater the risk of increasing TNF-α improvement level, and vice versa. In addition, the color of the point represents the improvement rate of TNF-α, and the darker the color, the better the effect. The results showed that, among the categorical variables, for respiratory, urinary, neurological and digestive diseases included in the "type of disease" feature, icariin and its derivatives had a more positive effect on the TNF-α efficacy of these systemic diseases compared with the baseline values. Meanwhile, "Wister mice" and "BALB/c mice", as species in the "Animal species" characterization, contributed more to the TNF-α efficacy compared with the baseline values. And "female" as a variable in the "sex" characteristic was also beneficial in improving the efficacy. (Figure 10B). As for continuous variables, when the "course" was greater than 31.22 days or the "dose" was greater than 27.52 mg/kg/day, icariin and its derivatives contributed more positively to the rate of improvement in TNF-alpha compared to baseline values (Figure 10C).

4 Discussion

4.1 Summary of the evidence

This investigation comprised the primary meta-analysis of icariin and its derivatives in the management of inflammation, accompanied by a machine learning analysis on the sample dataset. In total, 19 studies were incorporated, with 370 animals included in the meta-analysis and 260 animals analyzed through machine learning techniques. The results demonstrated that icariin and its derivatives possess the potential to treat many diseases by decreasing levels of cytokines via regulating related pathways.

4.2 Possible mechanism

Inflammation, as a complex and comprehensive process, plays key role in the emergence and development of variety diseases, such as cancer(Diakos, Charles, McMillan, & Clarke, 2014), acute kidney injury(Poston & Koyner, 2019), AS(Wolf & Ley, 2019), stroke(Lambertsen, Finsen, & Clausen, 2019), etc. Multiple cells, cytokines and pathways are involved in the process of inflammation. The pivotal step in the inflammatory response is the infiltration of immune cells, like macrophage, neutrophil. Activated immune cells secret various cytokines such as IL-1 and TNF-α, and facilitate the activation, proliferation and differentiation of T cells, B cells(Opal & DePalo, 2000). Simultaneously, the release of chemokines by macrophages also directs the movement of monocytes and neutrophils towards the site of inflammation, thereby amplifying the inflammatory response (Figure 11).

Research has indicated a strong correlation between inflammation and the onset of AS. The formation of foam cells in the arterial wall is a crucial aspect of AS, and it is closely linked to macrophages' phagocytosis and accumulation of cholesterol(Ding et al., 2021; D. Wang et al., 2019). SR-BI (Scavenger Receptor Class B Type I), a high-density lipoprotein transport protein(Yesilaltay, Kocher, Rigotti, & Krieger, 2005), can facilitate the uptake of HDL and enhance the reverse transport of cholesterol, thereby inhibiting foam cell formation(Chistiakov, Melnichenko, Myasoedova, Grechko, & Orekhov, 2017; Y. Xu et al., 2021). Notably, icariin has been shown to increase the expression of SR-BI protein, promoting HDL uptake and cholesterol reverse transport, which in turn suppresses macrophage cholesterol accumulation and foam cell formation, ultimately leading to therapeutic benefits for AS(H. Yang et al., 2015). And Cluster of Differentiation 36 (CD36), as a membrane receptor, can also mediate the transport of cholesterol and oxidize low-density lipoprotein, so it is involved in the occurrence and development of AS(Maréchal et al., 2018; Tian, Xu, Sahebkar, & Xu, 2020; R. Yang, Liu, & Zhang, 2022). At the same time, P38 mitogen-activated protein kinase pathway (P38 MAPK) can promote the activation of CD36(Maimaitiyiming, Zhou, & Wang, 2016). Icariin derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS Icariin machine derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS Icariin machine derivatives can block P38 MAPK and then inhibit CD36 activity, thereby

inhibiting the process of AS Icariin machine derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS Icariin machine derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS Icariin machine derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS Icariin machine derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS machine derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS derivatives can block P38 MAPK and then inhibit CD36 activity, thereby inhibiting the process of AS(H. Yang et al., 2015). As an inflammatory disease, myocarditis is regulated by various pathway mechanisms, and NF-ϰB is an important inflammatory signal transduction factor, which plays an important role in the occurrence and development of myocarditis(Yao et al., 2022). However, some studies have shown that Sirtuin 6 (SIRT6) down-regulates the expression of NF-ϰB and reduce myocardial inflammation by inhibiting the activation and nuclear translocation of NF-ϰB(Z. Jin et al., 2023; Song et al., 2020). Icariin up-regulate the level of SIRT6 to inhibit the activity of NF-ϰB and down-regulate the levels of IL-2, IL-6, TNF-α, ICAM-1 and other inflammatory factors to reduce myocardial inflammation(Y. Chen et al., 2015). The JNK pathway plays an important role in many biological processes including inflammation(H. Zhu et al., 2022). As the main transcriptional component of JNK, c-Jun, plays a role in both apoptosis and inflammation(H. Guo et al., 2020). However, it is worth noting that NF-ϰB can enhance the activity of c-Jun, thereby promoting the inflammatory process(Blonska & Lin, 2009). However, some studies have found that icariin can inhibit the activity of c-Jun by inhibiting the activation and release of NF-ϰB, thereby alleviating inflammation(H. Zhou et al., 2015). The study suggests that TGF-β can promote the transformation of cardiac fibroblasts into myofibroblasts and the synthesis of collagen by activating Samd2 phosphorylation in the heart.(Z.-G. Ma, Yuan, Wu, Zhang, & Tang, 2018). Icariin and its derivatives can down-regulate the level of TGF-β factor and inhibit the activity of Samd2, thereby inhibiting collagen synthesis and blocking the nuclear translocation and expression of NF-ϰB. It is worth noting that the nuclear translocation and expression of NF-ϰB can also be achieved through the inhibition of IϰB phosphorylation by ICS II(Fu et al., 2018). Ultimately achieve the purpose of treating myocardial inflammation.

Inflammatory processes are also present in many urological diseases. Nucleotide-binding domain and Leucine-rich Repeat containing family, Pyrin domain-containing 3 (NLRP3), as a component of inflammasome, can regulate the level of downstream inflammatory factors, and NLRP3 is also regulated by NF-ϰB(de Carvalho Ribeiro & Szabo, 2022; Sharma & Kanneganti, 2021; Q. Wang et al., 2020). In lupus nephritis (LN) and IgA nephropathy (IgAN), icariin can down-regulate the level of NLRP3 by inhibiting the activation of NF-ϰB, thereby reducing the levels inflammatory factors(Su et al., 2018; L. Zhang et al., 2017). Apoptosis-associated speck-like protein containing a CARD (ASC) is also an inflammasome that promotes the conversion of pro-caspase-1 to caspase-1 in IgAN to increase the level of inflammatory factors such as IL-1β(Hornung et al., 2009; Huang, Xu, & Zhou, 2021). Icariin and its derivatives can inhibit the activity of ASC, and then block the conversion process of pro-Caspase-1 to Caspase-1, then improve the level of inflammatory factors and treat inflammation(L. Zhang et al., 2017). As a protein kinase, IKKβ can phosphorylate IϰBα to release NF-ϰB and promote the production of inflammatory factors(Antonia, Hagan, & Baldwin, 2021; X. Dong et al., 2022). However, it is worth noting that icariin can delay this process by inhibiting the activity of IKKβ, so as to reduce the damage of IgAN to the kidney. At the same time, icariin can also treat IgAN by reducing the deposition of immunoglobulin A (IgA) and the expansion of mesangial matrix(L. Zhang et al., 2017). In nephrotoxicity and acute kidney injury, NF-ϰB is often inhibited by icariin as a key node, thereby improving the levels of downstream inflammatory factors such as TNF-α and inducible nitric oxide synthase (iNOS)(P. Ma et al., 2015; Xie et al., 2018).

In neurological diseases, inflammatory mechanisms assume an important role. In cerebral ischemia-reperfusion injury, studies have shown that PPARα and PPARγ can down-regulate NF-ϰB inflammatory pathways by inhibiting IϰBα phosphorylation(Collino et al., 2006; Q. Li et al., 2019), while icariin derivatives (IRS and ICS II) can increase High PPARα and PPARγ levels further inhibit related inflammatory pathways to achieve anti-inflammatory effects(Y. Deng et al., 2016; Xiong et al., 2016). As a transcription factor, Nrf2 can increase the level of superoxide dismutase (SOD) and reduce the generation of reactive oxy-

gen species (ROS) in neurodegenerative diseases (ND), thereby inhibiting NF-ϰB and MAPK inflammatory pathways activation(Ge et al., 2021; Jia, Zhang, Xu, Yao, & Wei, 2021; Kobayashi et al., 2016; L. Xu et al., 2018). And icariin can up-regulate Nrf2 level and reduce inflammatory response(L. Zhu et al., 2019). Experiments have shown that Phosphatidylinositol 3-kinase/Protein kinase B pathway (PI3-K/Akt pathway) can promote the release of inflammatory factors such as IL-1 and TNF-α in the process of nerve injury(C. Li, Zhao, Lin, Gong, & An, 2019). And existing studies have found that icariin can block the inflammatory process by inhibiting the PI3-K/Akt pathway(G. Q. Wang et al., 2017; H. Zhang et al., 2012).

In respiratory diseases, iNOS and Cyclooxygenase-2 (Cox-2), as two enzymes related to inflammatory response, can respectively promote the production of NO and Prostaglandin E2 (PGE2) when the body is stimulated by stimuli such as bacteria and viruses, and then inflammation is involved through a variety of inflammatory pathways(T. Li, Xu, Zhao, Gao, & Xie, 2022; Stiller & Hjemdahl, 2022). Studies have shown that icariin inhibits the activity of iNOS and Cox-2, thereby inhibiting the synthesis of related inflammatory mediators(C. Q. Xu et al., 2010). As for asthma, airway inflammation plays a pivotal role in its pathophysiological symptoms, which is mainly manifested in the infiltration of neutrophils and eosinophils(L. Dong et al., 2021). This process is regulated by various inflammatory factors including Interleukin-17 (IL-17)(Ritzmann, Lunding, Bals, Wegmann, & Beisswenger, 2022). And studies have shown that icariin significantly reduces airway inflammation via reducing the level of IL-17(Wei et al., 2015). Excessive secretion of mucus by mucous gland cells can also exacerbate airway inflammation, and icariin can also reduce this process(Jaramillo, Azzegagh, Tuvim, & Dickey, 2018). As an immune regulator, Forkhead box P3 (Foxp3) plays a key role in regulating the generation and differentiation of Regulatory T cells (Treg cells)(Y. Dong, Yang, & Pan, 2021). Interestingly, Retinoic acid receptor-related orphan receptor gamma t (RORγt) acts as a transcriptional Factors can bind to Foxp3 and reduce its activity, thereby inhibiting the development and function of Treg cells and relieving inflammation(W. Zhang et al., 2021). And icariin can just up-regulate the level of RORγt to inhibit the promoting effect of Foxp3 on the differentiation of Treg cells(Wei et al., 2015).

In inflammatory diseases of the digestive system, icariin and its derivatives exhibit multi-channel and multi-target characteristics in fighting inflammation. In colitis, Signal Transducer and Activator of Transcription 1 (STAT1) and Signal Transducer and Activator of Transcription 3 (STAT3) are two transcription factors present in Cluster of Differentiation 4 positive T lymphocytes (CD4+ T cells), which phosphorylation can promote the differentiation of CD4+ T cells into Th1 and Th17 cells, and then secrete inflammatory factors such as IFN-γ and IL-17 to promote inflammation(Celada et al., 2018; Kang, Biswas, Field, & Snapper, 2019; Kappel et al., 2009; Luz-Crawford et al., 2013). Icariin can inhibit the production of Th1 and Th17 cells by inhibiting the phosphorylation of STAT1 and STAT3, thereby alleviating the inflammatory effect(Tao et al., 2013). Furthermore, Cluster of Differentiation 25 (CD25) and Cluster of Differentiation 69 (CD69) are two molecules used to identify the state of T cell activation and proliferation(Shevach, McHugh, Piccirillo, & Thornton, 2001; Wiggins et al., 2022), among which CD25 can up-regulate the affinity of Interleukin-2 (IL-2) receptor(Codarri Deak et al., 2022), thereby guarantee the smooth flow of IL-2 pathway and promote the differentiation of T cells, while CD69 can increase the activity of T cells, prompt them to secrete inflammatory factors, and enhance cell membrane adhesion and other functions. Icariin inhibits the activity of CD25 and CD69 firstly, then inhibit the differentiation and function of T cells(Tao et al., 2013).

In diseases of the motor system, the occurrence of inflammation is also widely reflected. For osteolytic diseases, Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) can interact with Receptor Activator of Nuclear Factor Kappa-B (RANK) to promote the activation of osteoclasts and lead to osteolysis(Ikebuchi et al., 2018). Icariin can down-regulate the level of RANKL and inhibit the combination with RANK, thereby reducing the occurrence of osteolysis(Shao et al., 2015). As an enzyme, Cox-2 can promote the transformation of arachidonic acid into PGE2, and PGE2 can also promote the differentiation of osteoclasts(Zheng et al., 2019). Therefore, icariin prevents this transformation by inhibiting the activity of Cox-2 and reduces the differentiation of osteoclasts(Hsieh, Sheu, Sun, & Chen, 2011). It is worth noting that NF-ϰB pathway, P38 MAPK pathway, and JNK pathway can all stimulate the formation of osteoclasts and lead to osteolysis(An et al., 2019; Yin et al., 2019). And icariin can inhibit this process(Hsieh et al., 2011; J. Liu et al., 2016). In the related diseases of intervertebral disc degeneration, the P13K/AKT pathway can promote the

formation of bone matrix(D. Wang et al., 2022). Icariin does exactly that to intensify this process(X. Deng et al., 2017). Second, for osteitis and chondrocyte inflammation, elevated TNF-α levels can upregulate Matrix Metalloproteinase-1(MMP1), Matrix Metalloproteinase-1(MMP3), Matrix Metalloproteinase-1(MMP9), Matrix Metalloproteinase-1(MMP13), ROS levels, while promoting the degradation of IκBα and the nuclear translocation of NF-κB P65, resulting in inflammation(X. Chen et al., 2021; Kong et al., 2019; L. Liu & Kang, 2022). Icariin can inhibit the level of TNF-α to alleviate this process(M. H. Liu, Sun, Tsai, Sheu, & Chen, 2010; Mi et al., 2018; Pan, Zhang, Chen, & Yang, 2017).

According to research, tumor progression associated with chronic inflammation closely(Manjili, Isbell, Ghochaghi, Perkinson, & Manjili, 2022). Myeloid-derived suppressor cells (MDSCs) can prevent the activation of adaptive immunity to help tumor growth, and MDSCs are affected by chronic inflammation, specifically by upregulating Toll-like receptor 4 (TLR4) and MRP8/14 induced increased activity of MDSCs(H. Liu et al., 2021; Y. Wang, Ding, Guo, & Wang, 2019). Interestingly, ICA and ICT can downregulate the levels of TLR4 and MRP8/14 to inhibit the activation of MDSCs, thereby delaying tumor progression(J. Zhou et al., 2011).

4.3 Implications

Inflammation is a pathological stage of many diseases. Inflammation manifests in a variety of disease forms, and the currently selected therapeutic drugs are mainly non-steroidal anti-inflammatory drugs, glucocorticoids and other drugs. However, the application of anti-inflammatory effects of plant monomers is relatively limited. In addition, animal experiments to study the anti-inflammatory effect of monomers lack the results of effective clinical transformation, and there is also a lack of corresponding preclinical data collection. This article comprehensively utilizes the research on the treatment of inflammation with icariin, conducts meta-analysis based on its outcome index data to explore the curative effect, machine learning was then used to mine for more effective doses and courses of Icariin and its derivatives for the treatment of inflammation, and the types of inflammatory diseases for which they have an efficacy advantage. The results showed that icariin and its derivatives could improve TNF-α, IL-1β, IL-6, IFN-γ, TGF-β1, IkB-α, NF-κB p65, NLRP3, PPARα, SOD, MDA, BCL -2 and caspase-1 levels. And icariin and its derivatives have more favorable effect of inhibiting inflammation in respiratory, urinary, neurological and digestive diseases and at a dose of 27.52 mg/kg/day or more and a course of treatment of 31.22 days or more.

Animal experiments have always been an integral part of basic medical research, but laboratory animal research has always been subjected to ethical tests(Meier & Stocker, 1989). With the increase in the number of animal experiments and the expansion of the scale of a single experiment, the ethical review of the use of animals in experiments has become more stringent(Gruber & Hartung, 2004). How to efficiently select appropriate experimental animal models, treatment regimens and dosages of reagents, and make better use of animal experimental results, has gradually become an important issue(Loeb, Hendee, Smith, & Schwartz, 1989). Meta-analysis of animals can integrate published experimental results, save experimental resources, and obtain higher-level and more accurate preclinical evidence. At the same time, machine learning can be used to screen out features that are more important for drug treatment of diseases, so as to provide a reference for the selection of sample features for more in-depth animal experiments.

According to research, many drugs have shown good curative effects in the laboratory for treating diseases, but their clinical transformation rate is still low(van der Worp & Sandercock, 2012). Although there are many common mechanisms discovered in the laboratory, there are still differences in the organ structure between animals and humans, which cannot be ignored. Therefore, it is necessary to use machine learning to mine key features and select an appropriate animal model thereby increasing the clinical translation rate.

4.4 Limitations

(1) A total of 19 literatures were included in this study, all of which were in English, and only Su et al., Li et al., Xu et al., Tao et al., Guo et al., Wei et al. performed baseline Feature description. Xu et al., Shao et al., Deng et al. did not describe the blinding of performance bias. And the vast majority of literature, including Hu et al., reported on the existence of incomplete outcome data. (2) In the Egger's test for the

main indicators, although the results did not report the existence of publication bias, many hidden results will still affect the results of meta-analysis. (3) There is heterogeneity in the results of meta-analysis. After exploring the heterogeneity by means of subgroup analysis, the heterogeneity of some subgroups has been improved, but the rest of the subgroups still show some heterogeneity. The sources of these heterogeneities are very extensive, including but not limited to the experimental habits of researchers, laboratory light temperature environment, geographical latitude and magnetic field, etc. (4) Although the interpretability of the machine learning model has reached a high quality, the lack of sample data may still cause some trouble to the feature screening results.

5 Conclusion

Icariin and its derivatives have shown a significant improvement effect on inflammation-related diseases, and there is more favorable effect of inhibiting inflammation in respiratory, urinary, neurological and digestive diseases as well as when the dose is more than 27.52 mg/kg/day and course is more than 31.22 days. It has multiple therapeutic mechanisms including NF-κB pathway, P38 MAPK pathway, and PI3K/Akt pathway in different inflammatory diseases. Icariin and its derivatives may be a promising drug in the treatment of many diseases via inhibiting inflammation.

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Authors' contributions

Xiaochuan Guo and Yanqin Qin are the main contributors to this manuscript. Xiaochuan Guo performed the comprehensive summary and collation work. Yanqin Qin performed the literature search. Zhenzhen Feng, Haibo Li and Jingfan Yang and Caiyu Hu collected and organized graphical data. Sihan Hu and Junlin Li provided optimization suggestions for the article. Jiansheng Li (corresponding authors) conceived and coordinated the review. All authors read and approved the final manuscript.

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Table 1 The basic characteristics of the included literature

Study(year)

Disease

Species (sex, n = experimental

group for meta-analysis/model group, age, weight)

Model (method)

Treatment group

(drug, administration, dose, duration)

Model group

(administration, duration)

Outcome index

Wu et al. (2011)

Inflammation

C57BL mice (Female, 4/4, 8-10 weeks)

LPS((1mg/kg)

Gavage, ICT, 25/50/100mg/kg/d, 3 days

Gavage, H_2O, 3 days

Li et al. (2014)

Inflammation

BALB/c mice (Male, 10/10, 6 weeks)

Cigarettes (15 mg nicotine and 195 mg tar, 3 month)

Gavage, ICA,25/50/100mg/kg/d, 90 days

No procedures

Hu et al. (2016)

Atherosclerosis (AS)

Wistar rats (Male, 7/7, 180-220 g)

High-cholesterol diet (HCD, 83.8 % normal diet, 3.5 % cholesterol, 10 % animal oil, 0.2 % propylthiouracil, 0.5 % sodium cholate, and 5 % refined sugar, 9 weeks)

Gavage, ICA,30/60mg/kg/d, 28 days

No mention

Fu et al. (2018)

Myocardial fibrosis

Spontaneously hypertensive rats:SHRs(Male, 10/20, 13 weeks)

Spontaneously hypertensive rats

Gavage, ICS II, 4/8/16mg/kg/d, 84 days

Gavage, distilled water, 84 days

Su et al. (2018)

Lupus nephritis (LN)

MRL/lpr mice (10/10, 10 weeks)

MRL/lpr mice

Gavage, ICA, 10mg/kg/d, 56 days

Gavage, saline, 56 days

Zhang et al. (2017)

IgA nephropathy

SD rats (Male, 15/15, 6 weeks, 180-200 g)

0.1% BGG (8 weeks)

Gavage, ICA, 10mg/kg/d, 42 days

Gavage, saline, 42 days

Ma et al. (2015)

Acute renal injury

BALB/c mice (Male, 10/10, 20-22 g)

Cisplatin (15 mg/kg)

Gavage, ICA, 30/60mg/kg/d, 5 days

Gavage, saline, 5 days

Guo et al. (2010)

Brain dysfunction

SD rats (Male, 14/14, 10-14 weeks, 250-300 g)

LPS (10μl)

Gavage, ICA, 30/60/120mg/kg/d, 17 days

Gavage, distilled water, 17 days

Xiong et al. (2016)

Cerebral I/R

SD rats (Male, 5/5, 16 weeks, 250-280 g)

Reperfusion after MACO (24h)

Gavage, ICA, 10/30mg/kg/d, 3 days

Gavage, saline, 3 days

Deng et al. (2016)

Cerebral I/R

SD rats (Male, 5/5, 250-280 g)

Reperfusion after MACO (24h)

Gavage, ICS II, 10/30mg/kg/d, 3 days

Gavage, saline, 3 days

Li et al. (2018)

SCI

C57BL mice (Male, 6/6, 8 weeks)

Spinal cord after laminectomy

Gavage, ICA, 13.53/33.84mg/kg/d, 3 days

Gavage, saline, 3 days

Xu et al. (2010)

Acute lung inflammatory

C57BL mice (Male, 8/8, 11-12 weeks, 22-24 g)

LPS (5 mg/ kg)

Intraperitoneal injection, ICA, 20mg/kg/d, 0.25 days

Intraperitoneal injection, PBS (Phosphate Buffered Saline), 0.25 days

Wei et al. (2015)

Asthma

BALB/c mice (Female, 20/20,12-16 g)

3% OVA ovalbumin (21 days)

Gavage, ICA, 20/50/100mg/kg/d, 56 days

Gavage, saline, 56 days

Xie et al. (2018)

Acute kidney injury

C57BL mice (Male, 10/10, 38-44 weeks)

cecal ligation and perforation (CLP)

Intraperitoneal injection, ICA, 30/60mg/kg/d, 3 days

No mention

Zhang et al. (2015)

Intestinal I/R

SD rats (Male, 8/8, 180-220 g)

ischemiaereperfusion (I/R)

Gavage, ICA, 30/60mg/kg/d, 3 days

Gavage, 0.1% DMSO, 3 days

Shao et al. (2015)

Osteolysis

C57BL mice (Male, 21/21, 7-8 weeks)

20 mg Ti particle

Gavage, ICA,100/300mg/kg/d, 14 days

Gavage, saline, 14 days

Tao et al. (2013)

Colitis

C57BL mice (Female, 6/6, 8 weeks, 19-20 weeks)

2.5% dextran sulfate sodium DSS (7 days)

Gavage, ICA,3/10mg/kg/d, 10 days,

Gavage, drinking water, 10 days

Zhang et al. (2018)

Periodontitis

Mini pig (Female, 6/6)

Alveolar bone defects

Local injection, ICA,0.1 μg/mL, 84 days

Local injection, saline, 84 days

Zhou et al. (2011)

Tumor

BALB/c mice (Female, 5/5, 6-8 weeks)

Tumor inoculation

Intraperitoneal injection, ICA, 100mg/kg/d

No mention

Note:TNF-α IL-1β IL-6 TGF-β1 IkB-α PPARα PPARγ NF-κB p65 NLRP3 SOD MDA IFN-γ caspase-1 BCL-2 Bax

Ταβλε 2 Ρεσυλτς οφ ΤΝΦ-α συβγρουπ αναλψσις

Outcome parameters	Individuals (n)	SMD (9
(1) Subgroup analysis based on dose intervals	**(1) Subgroup analysis based on dose intervals**	**(1) Subg**
0-10mg/kg/day	$n = 32$	-3.393 (-5
11-20mg/kg/day	$n = 36$	-8.817 (-1
30-50mg/kg/day	$n = 12$	-6.745 (-9
51-100mg/kg/day	$n = 78$	-7.013 (-9
above 100mg/kg/day	$n = 70$	-6.159 (-1
(2) Subgroup analysis based on animal species	**(2) Subgroup analysis based on animal species**	**(2) Subg**
Rats	$n = 88$	-8.198 (-1
Mice	$n = 150$	-5.314 (-6
(3) Subgroup analysis based on drug duration	**(3) Subgroup analysis based on drug duration**	**(3) Subg**
0-5 days	$n = 72$	-6.471 (-7
5-10 days	$n = 12$	-2.524 (-4
11-20 days	$n = 70$	-6.159 (-1
20-50days	$n = 14$	-3.679 (-5
above 50 days	$n = 70$	-8.725 (-1
(4) Subgroup analysis based on sex	**(4) Subgroup analysis based on sex**	**(4) Subg**
Male	$n = 198$	-6.968 (-8
Female	$n = 20$	-5.171 (-1
NA	$n = 20$	-4.297 (-5
(5) Subgroup analysis based on type of diseases	**(5) Subgroup analysis based on type of diseases**	**(5) Subg**
Local inflammation	$n = 28$	-9.152 (-1
Circulatory system	$n = 44$	-8.271 (-1
Digestive system	$n = 28$	-4.923 (-9
Urinary system	$n = 40$	-5.616 (-8
Nervous system	$n = 40$	-8.078 (-1
Respiratory system	$n = 16$	-4.777 (-6
Motion system	$n = 20$	-3.571 (-4

Ταβλε 3 Ρεσυλτς οφ ΙΛ-1β συβγρουπ αναλψσις

Outcome parameters	Individuals (n)
(1) Subgroup analysis based on dose intervals	**(1) Subgroup analysis based on dose intervals**
0-10mg/kg/day	$n = 50$
10-20mg/kg/d	$n = 20$
21-30mg/kg/day	$n = 20$
31-50mg/kg/day	$n = 12$
51-100mg/kg/day	$n = 20$
above 100mg/kg/day	$n = 70$
NA	$n = 12$
(2) Subgroup analysis based on animal species	**(2) Subgroup analysis based on animal species**
Rats	$n = 98$
Mice	$n = 94$
(3) Subgroup analysis based on duration of treatment	**(3) Subgroup analysis based on duration of treatme**
0-5 days	$n = 52$
11-20 days	$n = 70$
20-50days	$n = 30$
above 50 days	$n = 40$
NA	$n = 12$
(4) Subgroup analysis based on sex	**(4) Subgroup analysis based on sex**
Male	$n = 172$
Female	$n = 12$
NA	$n = 20$
(5) Subgroup analysis based on type of diseases	**(5) Subgroup analysis based on type of diseases**
Local inflammation	$n = 12$
Circulatory system	$n = 50$
Urinary system	$n = 40$
Nervous system	$n = 60$
Motion system	$n = 42$

Table 4 Table of effects of Icariin and its derivatives on secondary indicators of inflammation, anti-inflammatory/transcriptional regulators, anti-oxidative stress indicators and apoptosis regulators

Outcome parameters	Experiments(n)	Indiv
(1) Secondary indicators of inflammation	**(1) Secondary indicators of inflammation**	**(1) Se**
IL-6	$n = 6$	n =16
IFN-γ	$n = 3$	$n = 3$
TGF-β1	$n = 5$	$n = 1$
IkB-α	$n = 5$	$n = 1$
NF-κB p65	$n = 2$	$n = 4$
NLRP3	$n = 2$	$n = 5$
(2) Anti-inflammatory/transcriptional regulators	**(2) Anti-inflammatory/transcriptional regulators**	**(2) A**
PPARα	$n = 2$	$n = 2$
PPARγ	$n = 2$	$n = 2$
(3) Anti-oxidative stress indicators	**(3) Anti-oxidative stress indicators**	**(3) A**
SOD	$n = 5$	$n = 8$
MDA	$n = 4$	$n = 6$
(4) Apoptosis regulators	**(4) Apoptosis regulators**	**(4) A**
BCL-2	$n = 4$	$n = 6$
caspase-1	$n = 2$	$n = 5$

Outcome parameters	Experiments(n)	Indiv
Bax	$n = 2$	$n = 3$

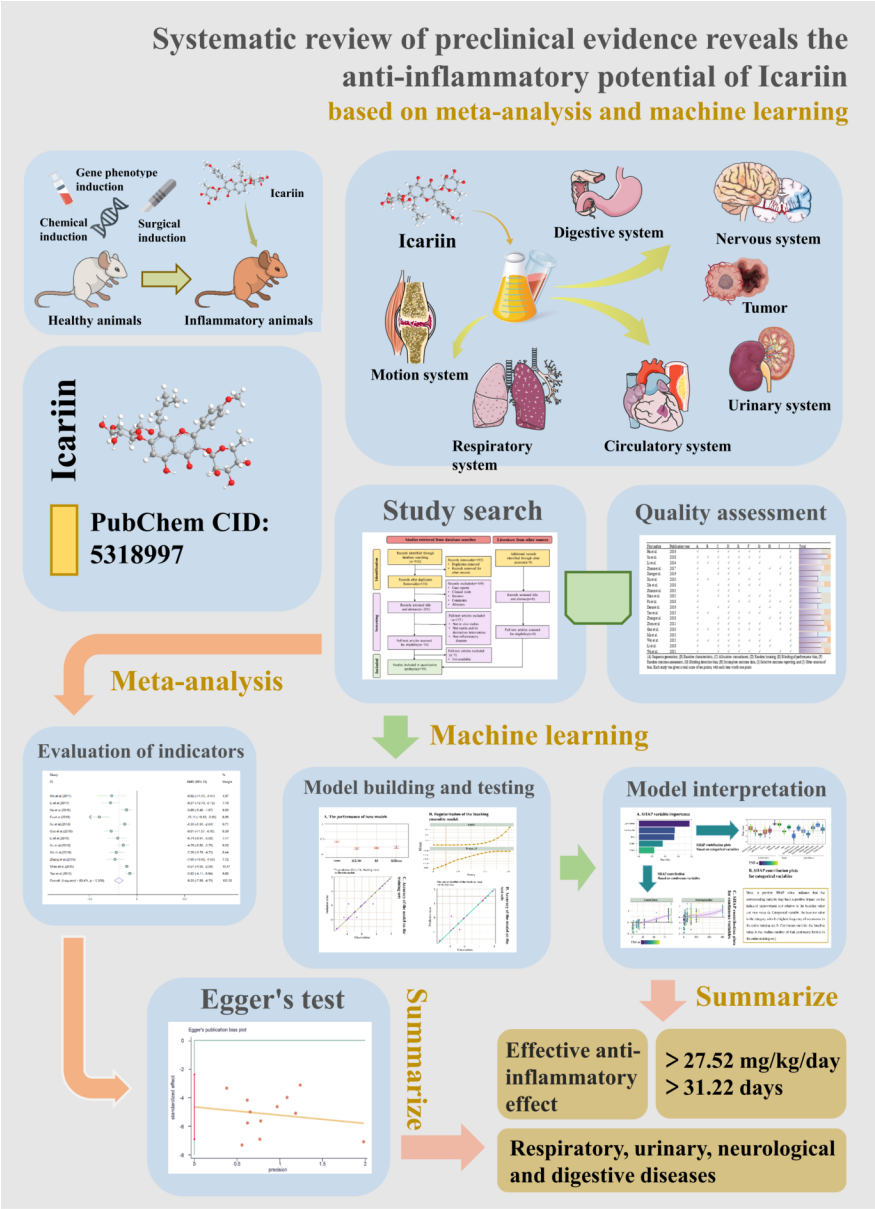


Figure1 Research roadmap

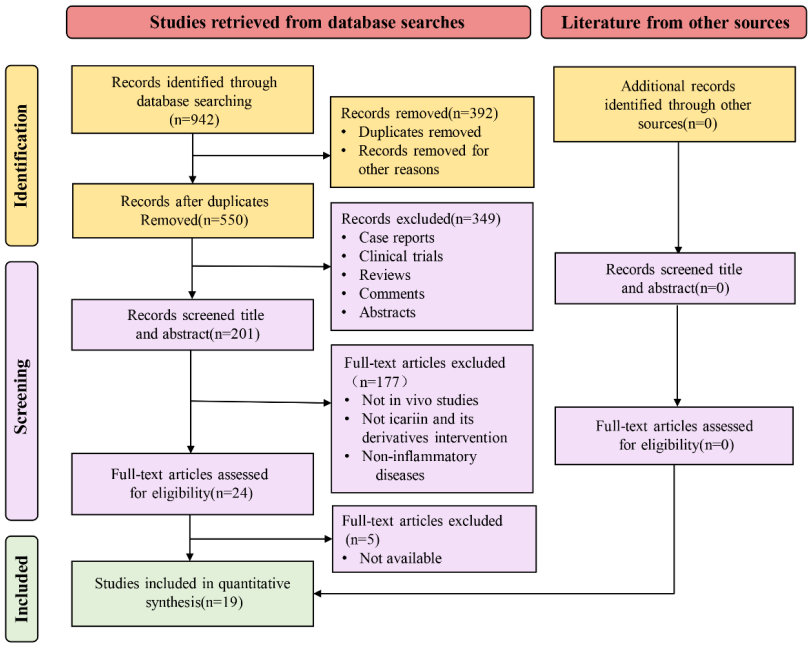


Figure 2 Flow diagram of the systematic review

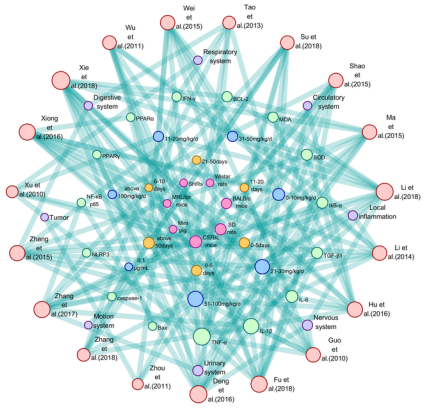


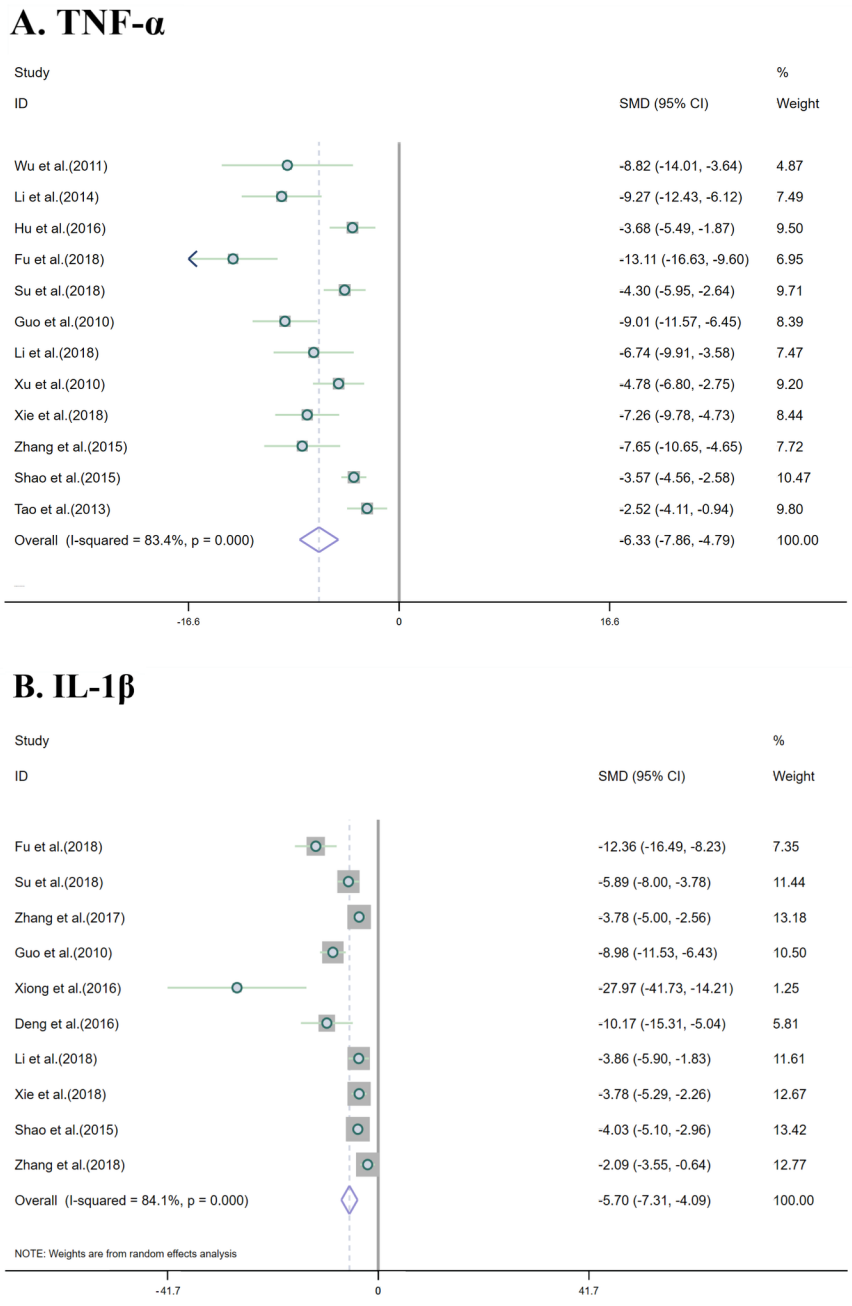
Figure 3 The basic characteristics of the included literature

(1. The variables represented by the network feature map from outer to inner are as follows: study, type of disease, outcome measures, dosage, duration of treatment, and species of experimental animals. 2. The size of the point represents the number of connected nodes, the greater the number of connected nodes, the larger the point.)

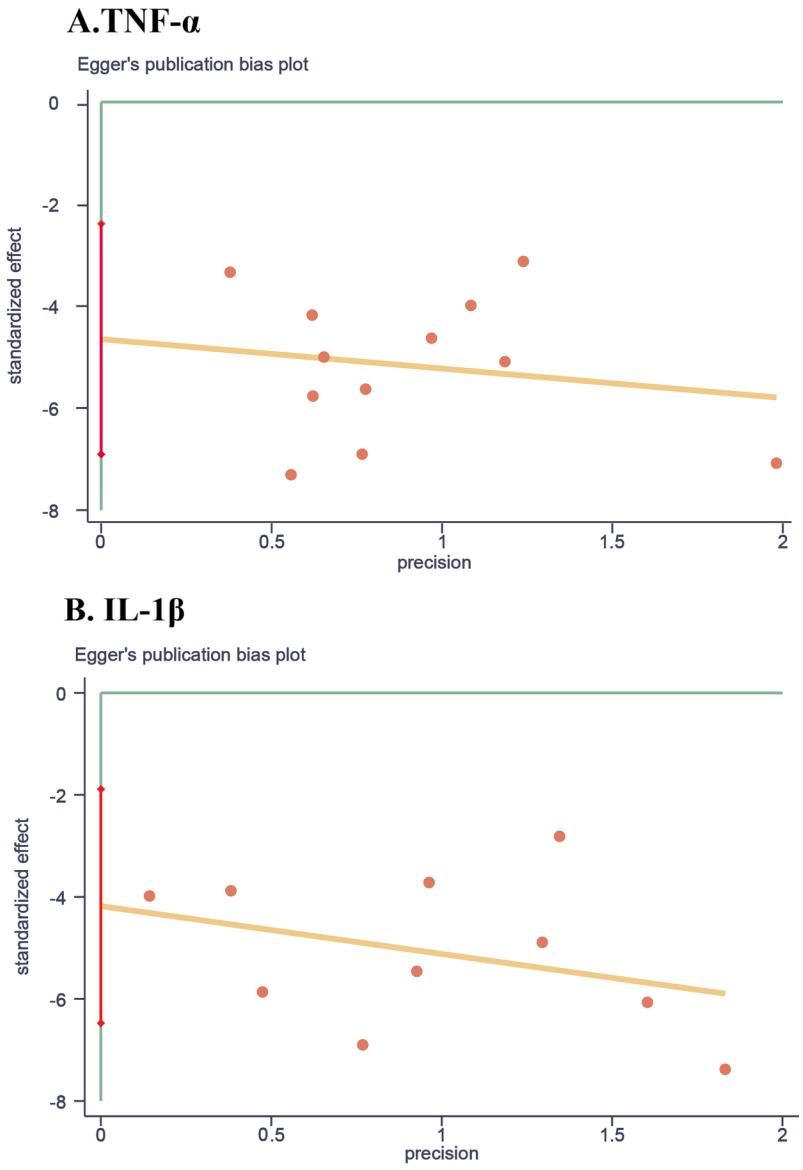
	Publication year	A	B	C	D	E	F	G	H	I	J	Total
Hu et al.	2016	√		√	√	√	√	√			√	
Su et al.	2018	√	√	√	√	√	√	√		√	√	
Li et al.	2014	√	√	√	√	√		√			√	
Zhang et al.	2017	√		√	√	√	√		√	√	√	
Xiong et al.	2016	√		√	√	√		√		√	√	
Xu et al.	2010	√	√		√		√	√			√	
Xie et al.	2018	√		√	√	√	√	√		√	√	
Zhang et al.	2015	√		√	√	√			√		√	
Shao et al.	2015	√		√	√		√	√	√		√	
Fu et al.	2018	√			√	√	√		√	√	√	
Deng et al.	2016	√		√	√			√	√		√	
Tao et al.	2013	√	√		√	√	√	√		√	√	
Zhang et al.	2018	√		√	√	√			√	√	√	
Zhou et al.	2011	√		√	√	√	√	√		√	√	
Guo et al.	2010	√	√	√	√	√	√	√		√	√	
Ma et al.	2015	√		√	√	√		√			√	
Wei et al.	2015	√	√	√	√	√	√	√	√		√	
Li et al.	2018	√		√	√	√		√	√		√	
Wu et al.	2011	√		√	√	√	√	√		√	√	

(A) Sequence generation; (B) Baseline characteristics; (C) Allocation concealment; (D) Random housing; (E) Blinding of performance bias; (F) Random outcome assessment; (G) Blinding detection bias; (H) Incomplete outcome data; (I) Selective outcome reporting; (J) Other sources of bias. Each study was given a total score of ten points, with each item worth one point.

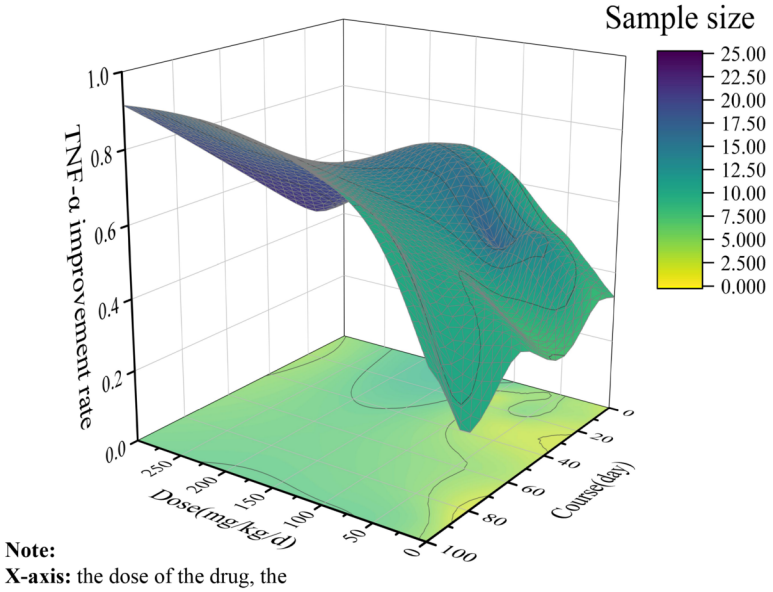
Figure 4 Risk of bias of included studies



Φιγυρε 5 Εφφεστς οφ Ισαριιν ον ινηιβιτινγ τηε ΤΝΦ-α, ΙΛ-1β (A)TNF-α (B)IL-1β



Φιγυρε 6 Πυβλιςατιον βιας βασεδ ον ΤΝΦ-α ανδ ΙΛ-1β (A) TNF-α· (B) IL-1β



Note:
X-axis: the dose of the drug, the
Y-axis: the course of the drug
Z-axis: TNF-α improvement rate: (experimental group - control group)/control group
Color mapping: sample size (color depth deepens with increasing sample size)

Φιγυρε 7 Τιμε-δοσε-εφφιςασψ ιντεραλ διστριβυτιον ωιτη ΤΝΦ-α ας ςυρατιε εφφεςτ στανδαρδ

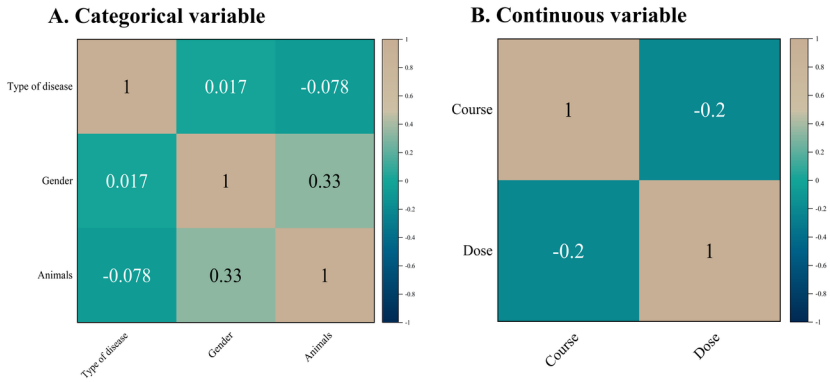


Figure 8 Correlation Between Independent Variables (A) Correlation Between Categorical Independent Variables. (B) Correlation between continuous independent variables.

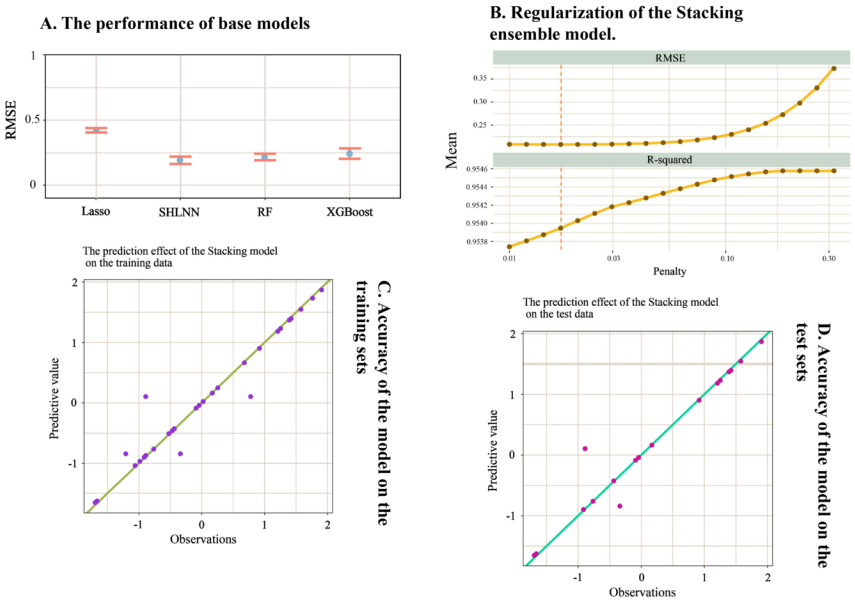


Figure 9 The performance of base models, regularization of the Stacking ensemble model, and the accuracy of the model on the dataset(A) The performance of base models. (B) Regularization of the Stacking ensemble model.

(C) Accuracy of the model on the dataset.

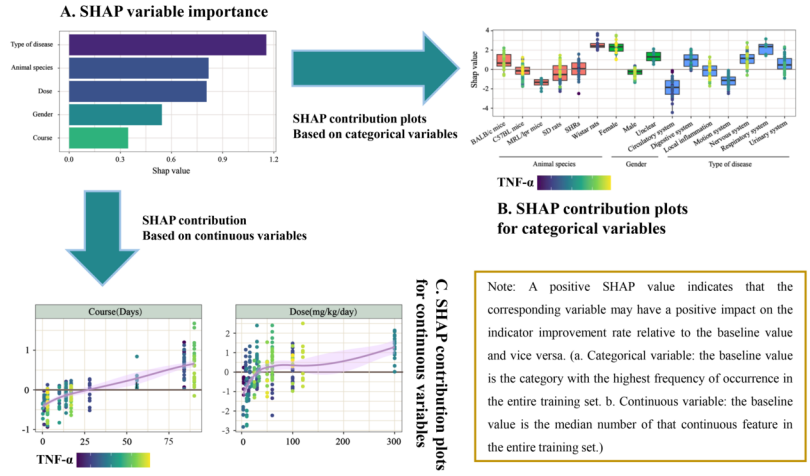


Figure 10 SHAP variable importance and SHAP contribution plots based on variable types (A) SHAP value variable importance map. (B) SHAP contribution plot based on categorical variables. (C) SHAP contribution plot based on continuous variable.

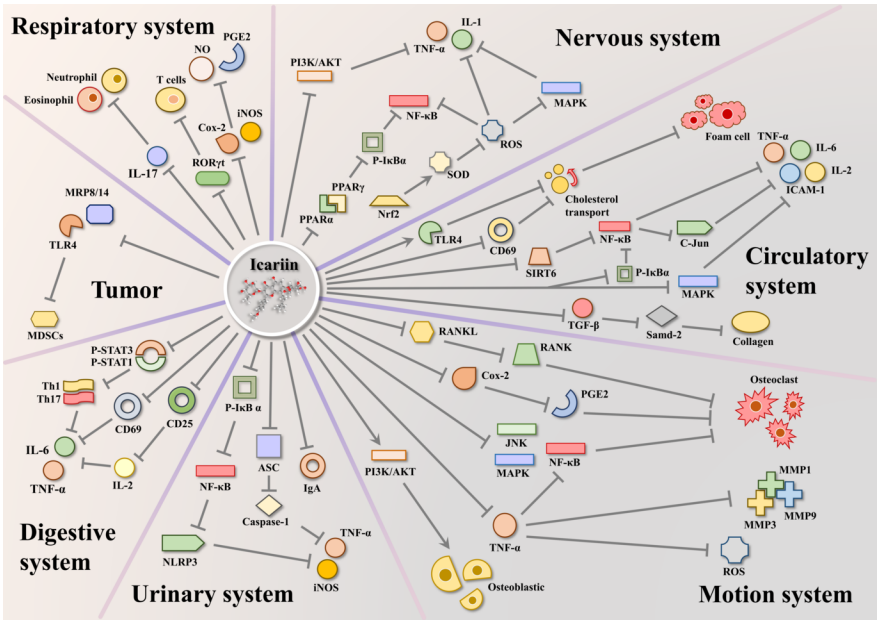
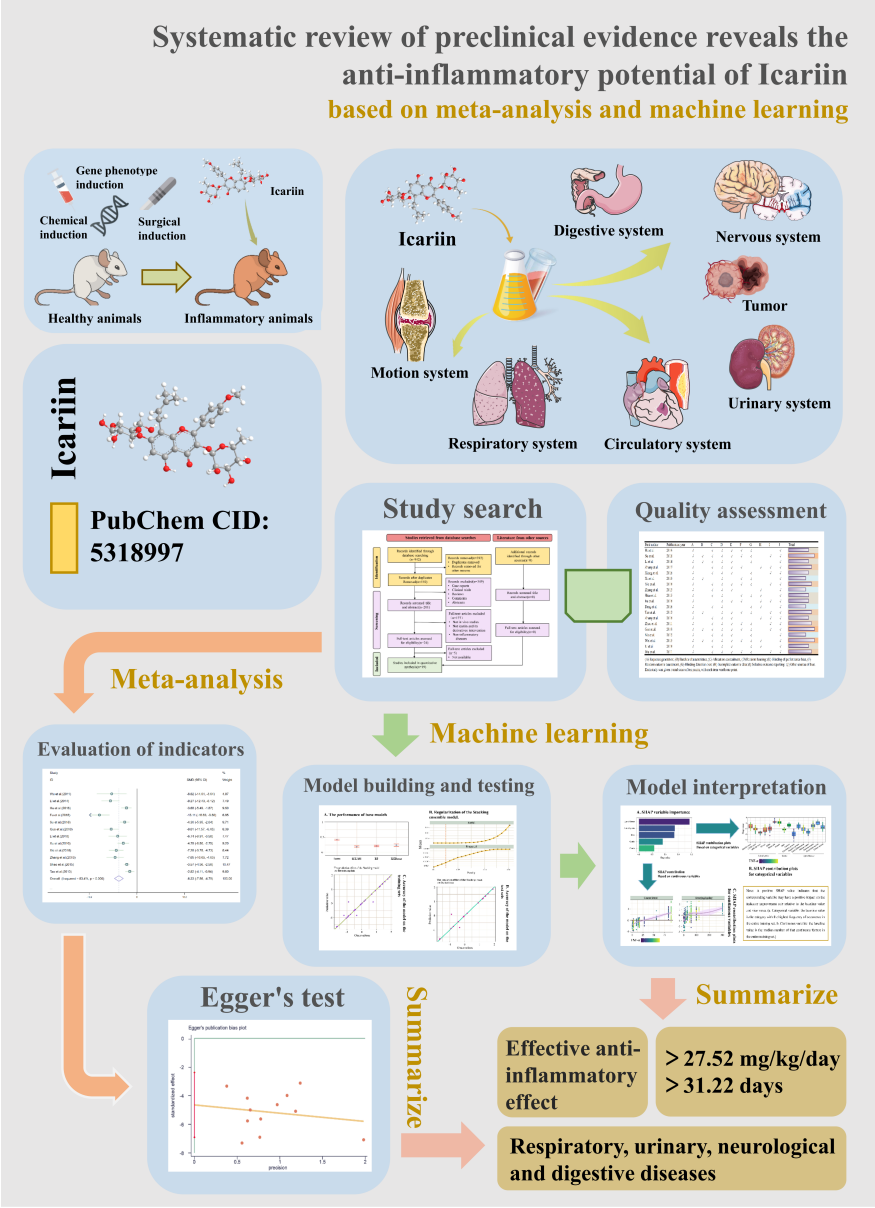
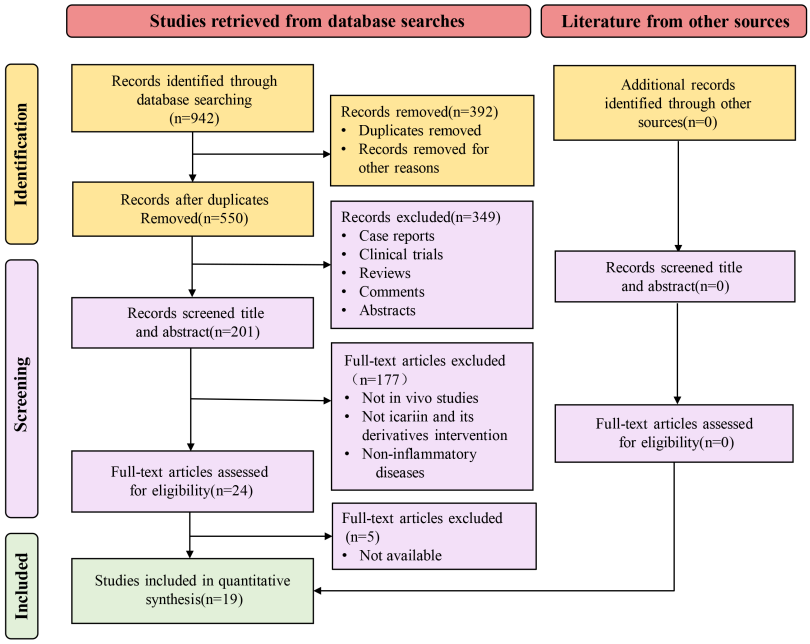


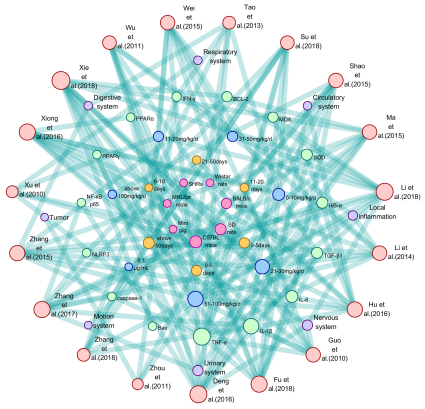
Figure 11 Icariin's potential anti-inflammatory mechanism

Hosted file

Supplementary File.docx available at https://authorea.com/users/651566/articles/659472-systematic-review-of-preclinical-evidence-reveals-the-anti-inflammatory-potential-of-icariin-based-on-meta-analysis-and-machine-learning



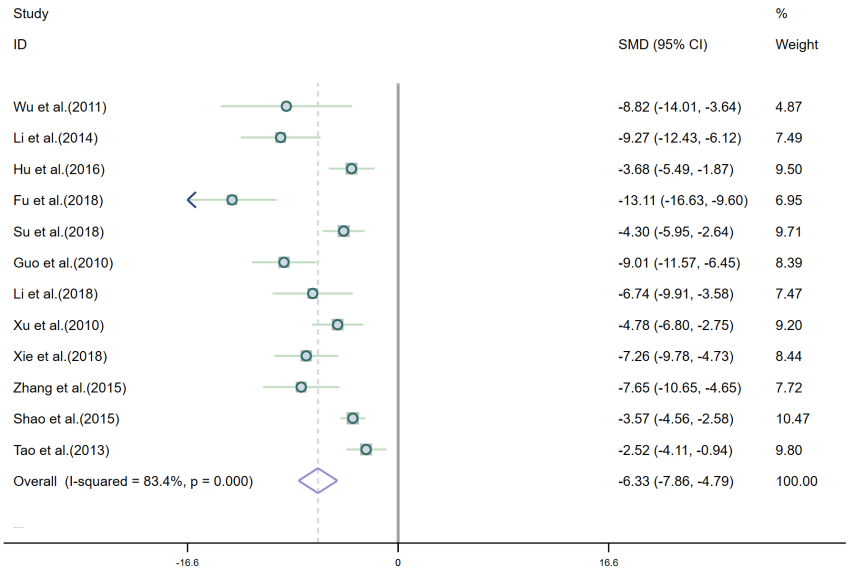




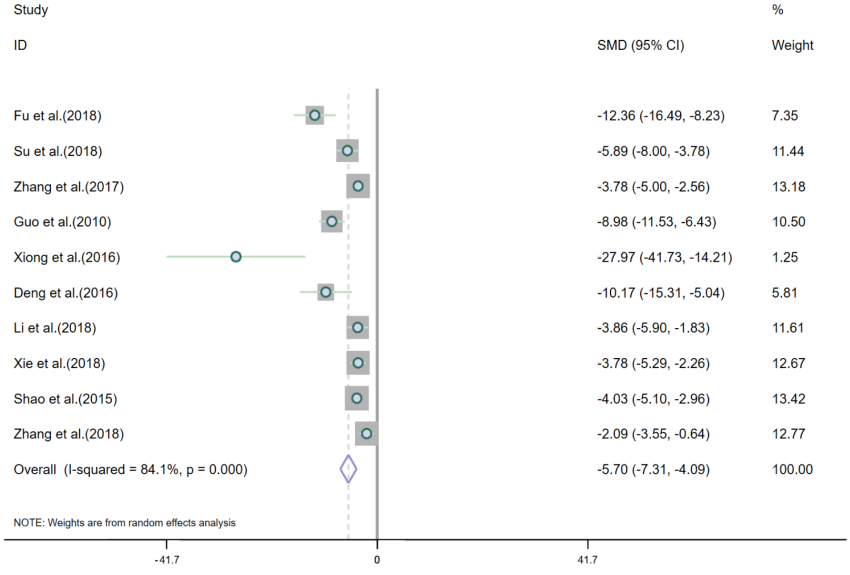
First author	Publication year	A	B	C	D	E	F	G	H	I	J	Total
Hu et al.	2016	√		√	√	√	√	√			√	
Su et al.	2018	√	√	√	√	√	√	√		√	√	
Li et al.	2014	√	√	√	√	√		√			√	
Zhang et al.	2017	√		√	√	√	√		√	√	√	
Xiong et al.	2016	√		√	√	√		√		√	√	
Xu et al.	2010	√	√		√		√	√			√	
Xie et al.	2018	√		√	√	√	√	√		√	√	
Zhang et al.	2015	√		√	√	√			√		√	
Shao et al.	2015	√		√	√		√	√	√		√	
Fu et al.	2018	√			√	√	√		√	√	√	
Deng et al.	2016	√		√	√			√	√		√	
Tao et al.	2013	√	√		√	√	√	√		√	√	
Zhang et al.	2018	√		√	√	√			√	√	√	
Zhou et al.	2011	√		√	√	√	√	√		√	√	
Guo et al.	2010	√	√	√	√	√	√	√		√	√	
Ma et al.	2015	√		√	√	√		√			√	
Wei et al.	2015	√	√	√	√	√	√	√	√		√	
Li et al.	2018	√		√	√	√		√	√		√	
Wu et al.	2011	√		√	√	√	√	√		√	√	

(A) Sequence generation; (B) Baseline characteristics; (C) Allocation concealment; (D) Random housing; (E) Blinding of performance bias; (F) Random outcome assessment; (G) Blinding detection bias; (H) Incomplete outcome data; (I) Selective outcome reporting; (J) Other sources of bias. Each study was given a total score of ten points, with each item worth one point.

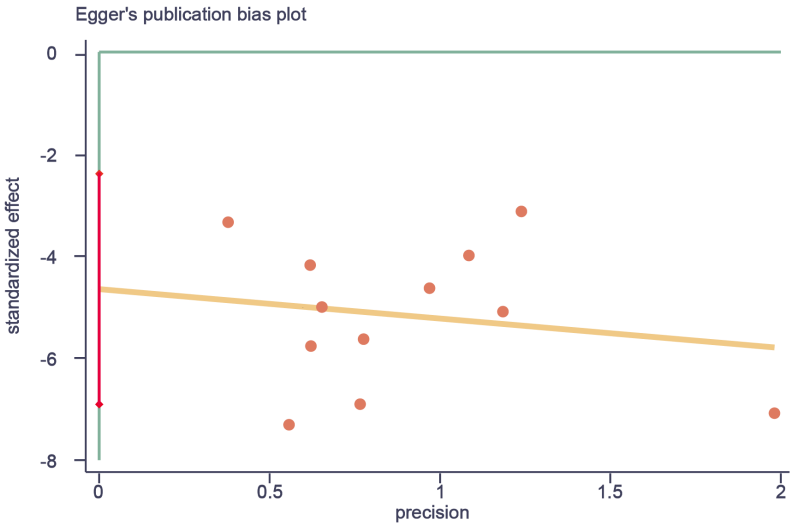
A. TNF-α



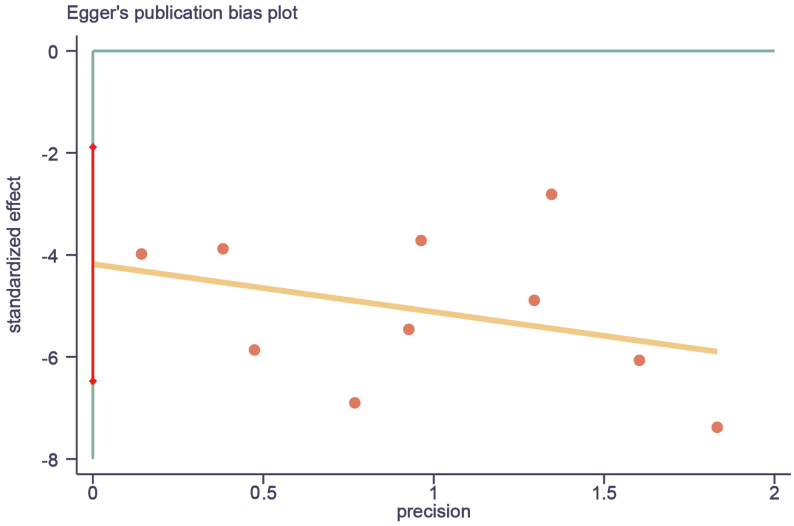
B. IL-1β

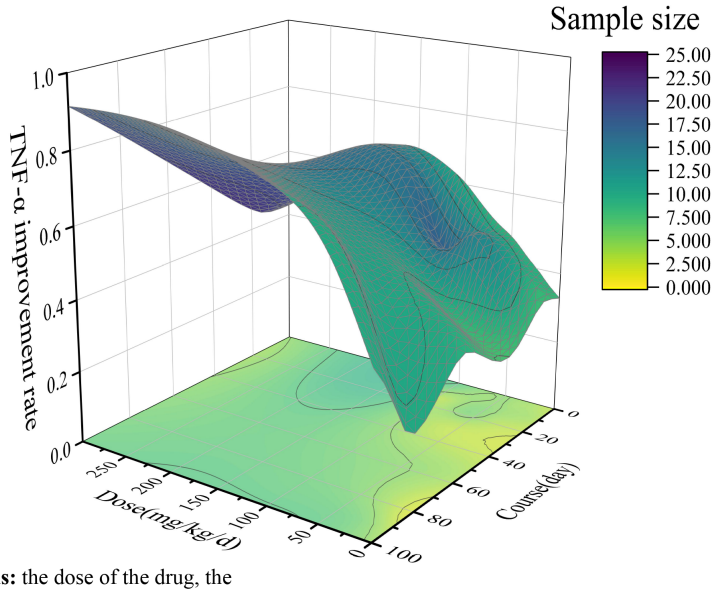


A.TNF-α



B. IL-1β





Note:
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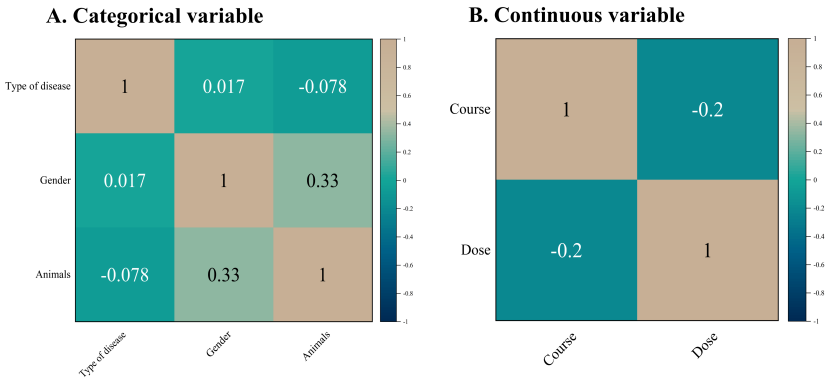

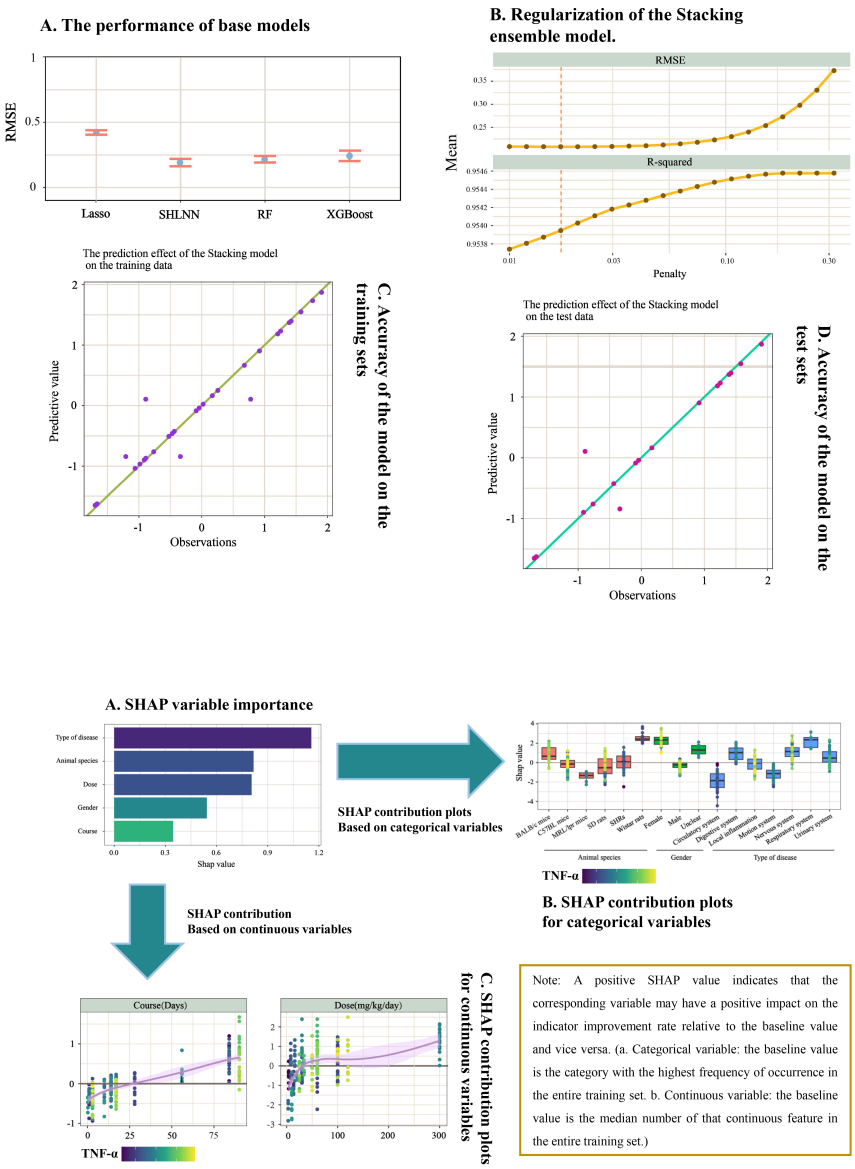

Note: A positive SHAP value indicates that the corresponding variable may have a positive impact on the indicator improvement rate relative to the baseline value and vice versa. (a. Categorical variable: the baseline value is the category with the highest frequency of occurrence in the entire training set. b. Continuous variable: the baseline value is the median number of that continuous feature in the entire training set.)