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Research article

Investigating the anti-inflammatory effects of icariin: A combined meta-analysis and machine learning study

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ABSTRACT

Objective: The objectives of this study were to define the superiority of icariin and its derivatives' anti-inflammatory activities and to create a reference framework for evaluating preclinical evidence. This method combines machine learning and meta-analysis to identify underlying biological pathways.

Methods: Data came from PubMed, Embase, Web of Science, and the Cochrane Library. SYRCLE was used to evaluate the risk of bias in a subset of research. Meta-analysis and detailed subgroup analyses, categorized by species, genders, disease type, dosage, and treatment duration, were performed using R and STATA 15.0 software to derive nuanced insights. Employing R software (version 4.2.3) and the tidymodels package, the analysis focused on constructing a model and selecting features, with TNF-α as the dependent variable. This approach aims to identify significant predictors of drug efficacy. An in-depth literature facilitated the synthesis of anti-inflammatory mechanisms attributed to icariin and its constituent compounds.

Results: Following a meticulous search and selection process, 19 studies, involving 370 and 260 animals were included in the meta-analysis and machine-learning assessment, respectively. The findings revealed that icariin and its derivatives markedly reduced inflammation markers, including TNF-α and IL-1β. Additionally, machine-learning outcomes, with TNF-α as the target variable, indicated enhanced anti-inflammatory effects of icariin across respiratory, urological, neurological, and digestive disease types. These effects were more pronounced at doses exceeding 27.52 mg/kg/day and treatment durations beyond 31.22 days.

Conclusion: Strong anti-inflammatory effects are exhibited by icariiin and its derivatives, which are especially beneficial in the management of digestive, neurological, pulmonary, and urinary conditions. Effective for periods longer than 31.22 days and at dosages more than 27.52 mg/kg/day. Subsequent research will involve more targeted animal experiments and safety assessments to obtain more comprehensive preclinical evidence.

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1. Introduction

Known as "Yin-Yang-Huo," Icariin (PubChem CID: 5318997) is a flavonol glycoside molecule that is extracted from the traditional Chinese medicinal herb Epimedium. It is highly valued for its diverse range of bioactive components [1]. Recently, icariin has emerged as a promising candidate for therapeutic intervention in a wide spectrum of diseases. Previous studies have established its efficacy in modulating inflammation [2], quenching oxidative stress [3], preventing apoptosis [4], and suppressing tumorigenesis [5].

Compared to other drugs and herbal monomers, icariin has demonstrated multifaceted potential in laboratory evidence. For instance, non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen are widely used to reduce inflammation but often target a single pathway, leading to significant gastrointestinal side effects and long-term cardiovascular risks [6]. Corticosteroids, such as prednisone, also target specific inflammatory pathways but are associated with adverse effects like immunosuppression, osteoporosis, and metabolic disturbances when used chronically [7]. Similarly, herbal monomers like baicalin, extracted from Scutellaria baicalensis, show significant anti-inflammatory properties but may exhibit limited bioavailability and rapid metabolism, reducing their therapeutic potential [8]. Curcumin, a component of Curcuma longa, also demonstrates potent anti-inflammatory effects but suffers from poor solubility and absorption, leading to challenges in achieving effective concentrations in vivo [9]. Resveratrol, found in Polygonum cuspidatum, exhibits anti-inflammatory activity but requires high doses to be effective, which can be impractical for clinical use [10]. Additionally, many of these herbal monomers have a limited scope of action, affecting specific pathways or systems.

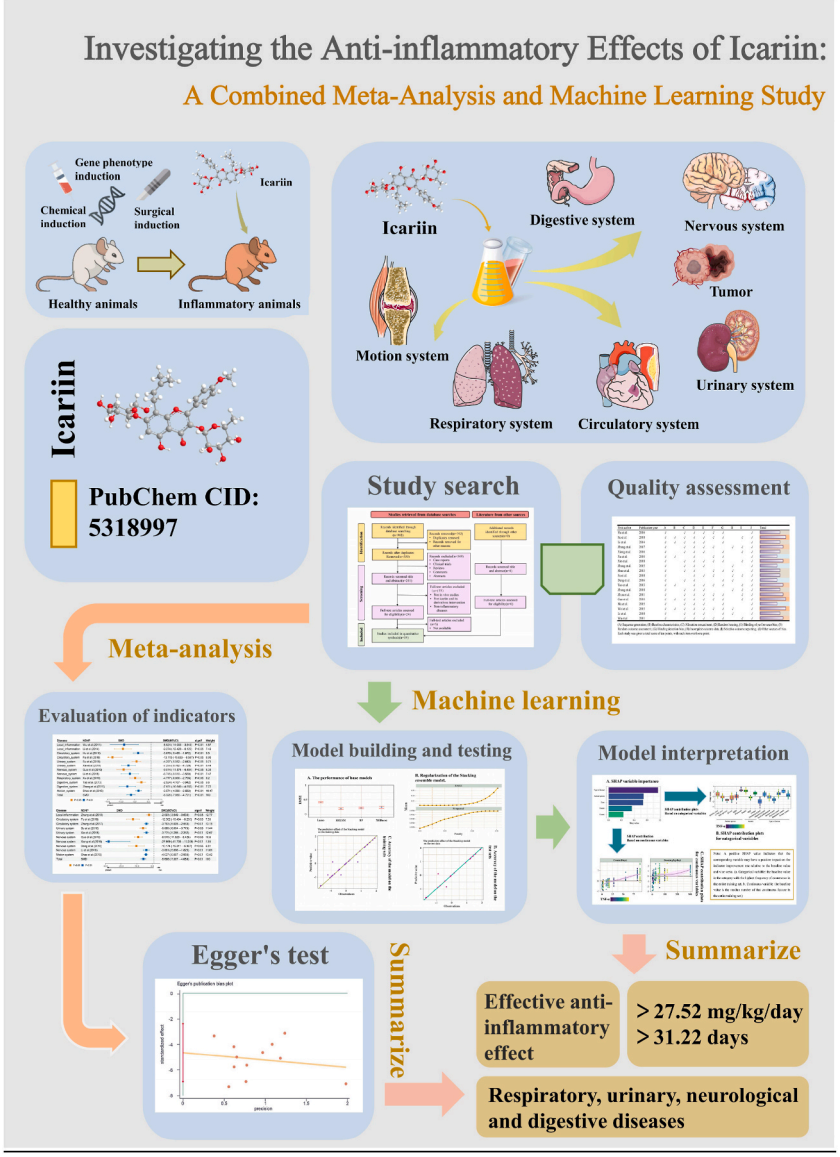


Fig. 1. Research roadmap.

In contrast, Icariin offers several distinct advantages. It provides a multi-faceted approach to inflammation modulation by targeting multiple signaling pathways, which can offer a broader and potentially more effective therapeutic profile. This multi-pathway mechanism reduces the likelihood of severe side effects associated with single-pathway drugs and enhances its therapeutic potential. Furthermore, Icariin exerts anti-inflammatory effects across various organ systems, including the respiratory, digestive, urinary, and neurological systems, highlighting its broad-spectrum activity. Additionally, Icariin has a well-documented safety profile and a history of use in traditional medicine, supporting its potential for safe and effective therapeutic applications.

Gathering evidence from extensive experiments is vital for confirming the effectiveness of new treatments. Yet, inconsistencies in in vivo studies and ethical concerns over animal use are notable challenges [11,12]. The synthesis of statistical data from various in vivo studies provides a solution. It provides a refined and, objective analysis and minimizes unnecessary animal testing. Systematic data reviews have accelerated drug development processes, with successful applications in treatments including the use of melatonin for cardiac injuries and quercetin for hepatic disorders [13,14]. Proper drug dosage and treatment schedules are essential, as they influence the results of animal studies results. Advancements in machine learning have facilitated the evaluation of drug effects across diseases, aiding in the design of preclinical and initial clinical trials. This included the determination of the optimal dosage and treatment periods [15]. In summary, integrating systematic reviews with machine learning boosts preclinical research productivity and translational potential. This approach also offers insights for future animal studies and enhances overall experimental effectiveness. The research plan is outlined as follows (Fig. 1).

2. Method

2.1. Meta-analysis

2.1.1. Search strategy

Major databases, including PubMed, Embase, the Cochrane Library, and Web of Science, were searched in order to find publications that had been published until December 2023, with no publication restrictions. Our search strategy involved using the phrase "treatment combined with disease" to locate pertinent research on the anti-inflammatory effects of icariin. During the database search, keywords associated with icariin, epimedium, Yin-Yang-Huo, and inflammatory diseases were utilized.

2.1.2. Study selection

The inclusion of the studies was independently assessed by two authors based on the established inclusion and exclusion criteria [14,16]. In cases of disagreement, a third party arbitrated to determine whether the literature should be included [17].

The studies included in the review adhered to the following criteria: (1) physical ligation and chemical induction methods were used to establish animal models for inflammatory diseases; (2) The animal models spanned various species, genders, ages, weights, and sample sizes without restriction; (3) icariin or its derivatives were the sole treatments for the experimental group, whereas the controls received vehicle or no intervention. The outcome assessment criteria included main and secondary indicators of inflammation, regulators of inflammation/transcription, anti-oxidative stress indicators, and apoptosis regulators, as shown in the following table (Table 1).

The review excluded the following: (1) editorial pieces, review articles, conference summaries, in vitro, and clinical investigations. (2) studies not focusing on diseases in inflammatory or non-inflammatory stages; (3) unpublished manuscripts; (4) repetitive publications.

2.1.3. Data extraction

Two researchers independently extracted data from the selected articles, focusing on (1) authors and year of publication; (2) details of the animal models, such as species, sex, and number of subjects; (3) techniques used to induce inflammation; (4) anesthesia methods; (5) information on intervention drugs including names, dosages, and schedules; and (6) details of the group drugs, with the highest dose used; (7) for graphically presented doses, the original authors were contacted or digital ruler software was used for image measurements.

Table 1
Outcome indicators.

Main indicators of inflammation	
Tumor Necrosis Factor-alpha (TNF-α)	Interleukin-1 beta (IL-1β)
Secondary indicators of inflammation	
Interleukin-6 (IL-6)	Interferon gamma (IFN-γ)
Transforming Growth Factor beta-1 (TGF-β1)	Inhibitor kappa B α (IkB-α)
NF-kappa-B (NF-κB) p65 subunit	Nucleotide-binding oligomerization domain (NLRP3)
Anti-inflammatory/transcriptional regulators	
Peroxisome Proliferator-Activated Receptor alpha (PPARα)	Peroxisome Proliferator-Activated Receptor gamma (PPARγ)
Anti-oxidative stress indicators	
Superoxide Dismutase (SOD)	Malondialdehyde (MDA)
Apoptosis regulators	
B-cell lymphoma-2 (BCL-2)	Bcl-2 Associated X protein (Bax)

2.1.4. Risk of bias assessment

The SYRCLE method was utilized to assess the potential for bias in research concerning the effects of icariin on inflammatory illnesses [18]. The assessment included 10 criteria: (1) generating sequences, (2) initial characteristics, (3) concealing allocations, (4) random housing, (5) blinding for performance bias, (6) random assessment of outcomes, (7) blinding for detection bias, (8) completeness of outcome data, (9) selective outcome reporting avoidance, and (10) other biases. The studies were scored on a ten-point scale, with one point per criterion. Discrepancies in the Risk of Bias Assessment were resolved by a third reviewer.

2.1.5. Statistical analysis

Measures of continuous outcomes were assessed using 95 % confidence intervals and the Standardized Mean Difference (*SMD*). To aggregate the impact estimates, a random effects model was employed, given the expected heterogeneity among the included studies [19]. A p-value of less than 0.05 indicated statistical significance between the experimental and control groups. The heterogeneity of the study was evaluated using the I-square (I^2) statistic. An I^2 value of 50 % or less indicated homogeneity among the included studies, whereas a value greater than 50 % suggested substantial heterogeneity, necessitating subgroup analyses to discern the underlying causes. Effect sizes across the studies were synthesized using random-effects models [20].

2.2. Machine learning

2.2.1. Selection of research objects and variables

The data satisfied the same inclusion and exclusion criteria and were taken from the literature that was part of the meta-analysis. It is crucial to acknowledge that varying sample sizes across studies may introduce potential biases that affect the machine learning outcomes. Data encompassing all the doses and durations were compiled for each study. Relevant sample data for each document were averaged for consistency. To fully explore the inhibitory effects of icariin dosage and treatment course on inflammation in different systems and the influence of animal species and genders on the anti-inflammatory effect, we used icariin dosage, treatment course, type of disease, and the species and genders of experimental animals as independent variables. At the same time, in order to more accurately measure the level of inflammation, we selected the TNF-α improvement rate, the most frequently included indicator in all studies, calculated as ((Mean of Control group - Mean of Experimental group)/Mean of Control group).

2.2.2. Data processing

The dataset used in this study comprised 260 samples characterized by significant discreteness. Data standardization (shifting the sample mean to 0 and standard deviation to 1) offers benefits such as a more focused data distribution, removal of dimensional disparities among data, and enhanced interpretability of machine-learning models [21]. Consequently, we applied data normalization to our dataset to refine the structure.

2.2.3. Development and assessment of predictive models

Based on the continuous nature of the dependent variable, we selected four models suitable for regression prediction tasks as foundational models to predict TNF-α improvement levels. These models included: Extreme Gradient-Based Optimization (XGBoost), Random Forest (RF), Linear Absolute Shrinkage and Selection Optimization (Lasso), and Simple Hidden Layer Neural Network (SHLNN). These models were chosen based on their proven effectiveness in handling similar types of data and prediction tasks. Data standardization was utilized for optimization, along with a 5-fold cross-validation ($v = 5$), employing 10 random partitions per fold ($break = 10$) as a resampling technique. Subsequently, the models were established, their hyperparameters were fine-tuned using a grid search, and their performance was evaluated through cross-validation. Once constructed, these models were integrated into the Stacking Model as basic-features. Lasso was then applied to these meta-features to refine the model, which was subsequently deployed for the prediction and assessment of both the training and test sets. Model evaluation was conducted using the R-squared (R^2), Root Mean Squared Error (*RMSE*), and Mean Absolute Error (*MAE*) as metrics.

2.2.4. Model interpretation

Although interpreting machine-learning models can be complex, the SHapley Additive explanations (SHAP) approach, developed by Lundberg et al. [22] and grounded in game theory, offers a solution by providing precise model output interpretations. The input characteristics' significance was evaluated by the SHAP values, which also showed how they affected the model's prediction. A greater SHAP number signifies a noteworthy positive impact, whereas a lower value denotes a minimal or negative influence.

2.3. Analysis software and version

Python (version 3.11.3), R (version 4.2.3), and STATA (version 15.0) were used in this study.

3. Results

In all, 942 publications were found during the initial literature search. After removing duplicates, 550 unique articles remained. Subsequently, a comprehensive assessment of the titles and abstracts led to the selection of 201 articles for an in-depth full-text review. A meticulous examination of the complete texts resulted in the inclusion of 19 articles for the analysis, with 182 articles being excluded (not in vivo studies, not using Icariin and its derivatives, non-inflammatory diseases, lack of outcome indicators) [23–41] (Fig. 2).

3.1. Characteristics of the included studies

We examined 370 animals from 19 English-language publications, 180 of which were in the experimental group and 190 of which were in the control group (Fig. 3A). The investigated inflammatory conditions covered various areas, including local, circulatory, urinary, nervous, respiratory, digestive, motion, and tumor-related inflammation (Fig. 3B). The study utilized diverse animal models such as Sprague-Dawley rats, C57BL27 mice, BALB/c mice, Wistar rats, MRL/lpr mice, and minipigs (Fig. 3C). With a rigorous emphasis on disorders related to inflammation, animal models were chosen using predetermined inclusion and exclusion criteria. In contrast to the control group, which was given regular saline or distilled water as treatment, the experimental group got icariin or its derivatives as pure medication. The evaluation of icariin's effects on inflammatory diseases noted biomarkers such as TNF-α, Interleukin-1 beta cytokine (IL-1β), Transforming Growth Factor subtype beta-1 (TGF-β1), Superoxide dismutase enzyme (SOD), Malondialdehyde (MDA), B-cell leukemia 2 (BCL-2), Interferon gamma (IFN-γ), Bcl-2-Associated X-protein (Bax), Caspase-1, Peroxisome Proliferator-Activated Receptor alpha (PPARα) and gamma (PPARγ), Nucleotide-binding oligomerization domain (NLRP3), and NF-kappa-B (NF-κB) p65, each mentioned in at least two studies (Fig. 3D) (Table 2).

3.2. Study quality

19 articles total, all of which described the randomization of animals into experimental and control groups were examined. The animal features and prognostic variables were covered in 6 articles. Additionally, the methodologies for allocation concealment were detailed in 16 articles, with all studies clarifying random room allocation for animals. The blinding of the trial participants was reported in 16 articles. Moreover, 12 articles detailed the random selection process for outcome assessment and 15 articles mentioned blinding during this phase. Moreover, information pertaining to experimental attrition and sample exclusion was transparently reported in 8 articles, while the completeness of the data was explicitly addressed in 10 articles. Notably, within these 19 articles, no discernible sources of bias or indications for selective reporting were identified (Fig. 4).

3.3. Efficacy and mechanism of meta-analysis

3.3.1. Primary inflammatory markers

3.3.1.1. TNF-α. The analysis of 12 studies revealed a notable decrease in TNF-α levels with icariin and its derivatives [$n = 238$, $p = 0.000$, $SMD = -63.26$ (-7.860, -4.791)] (Fig. 5A). High heterogeneity was detected among the studies ($I^2 = 83.4$ %), which led to subgroup analyses. These analyses were based on variables such as dose, treatment duration, disease type, species, and sex of the animals. Improvements in heterogeneity were significant in subgroups of "0–5 days" (course) and "Local inflammation" and "Nervous system" (type of diseases), highlighting these factors as major sources of heterogeneity (Table 3).

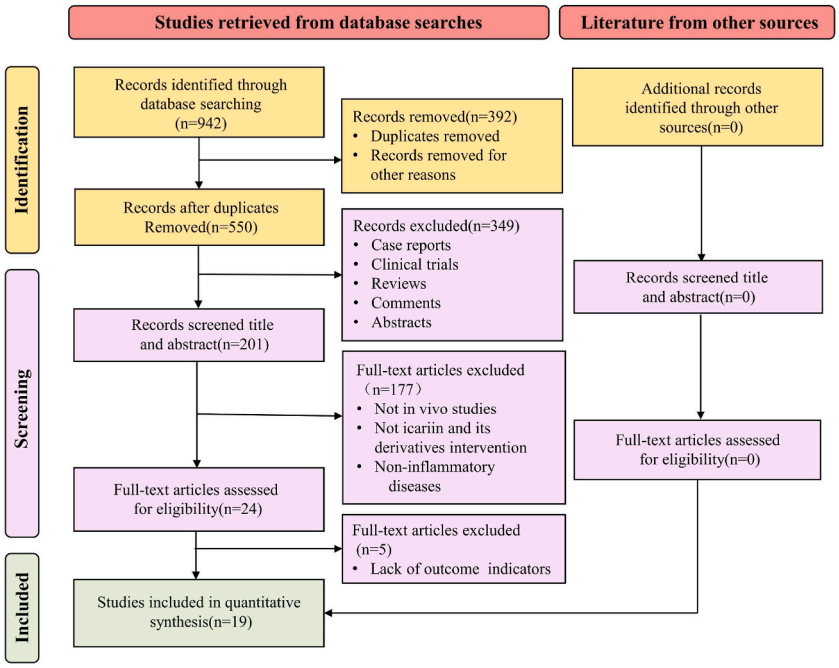


Fig. 2. Flow diagram of the systematic review.

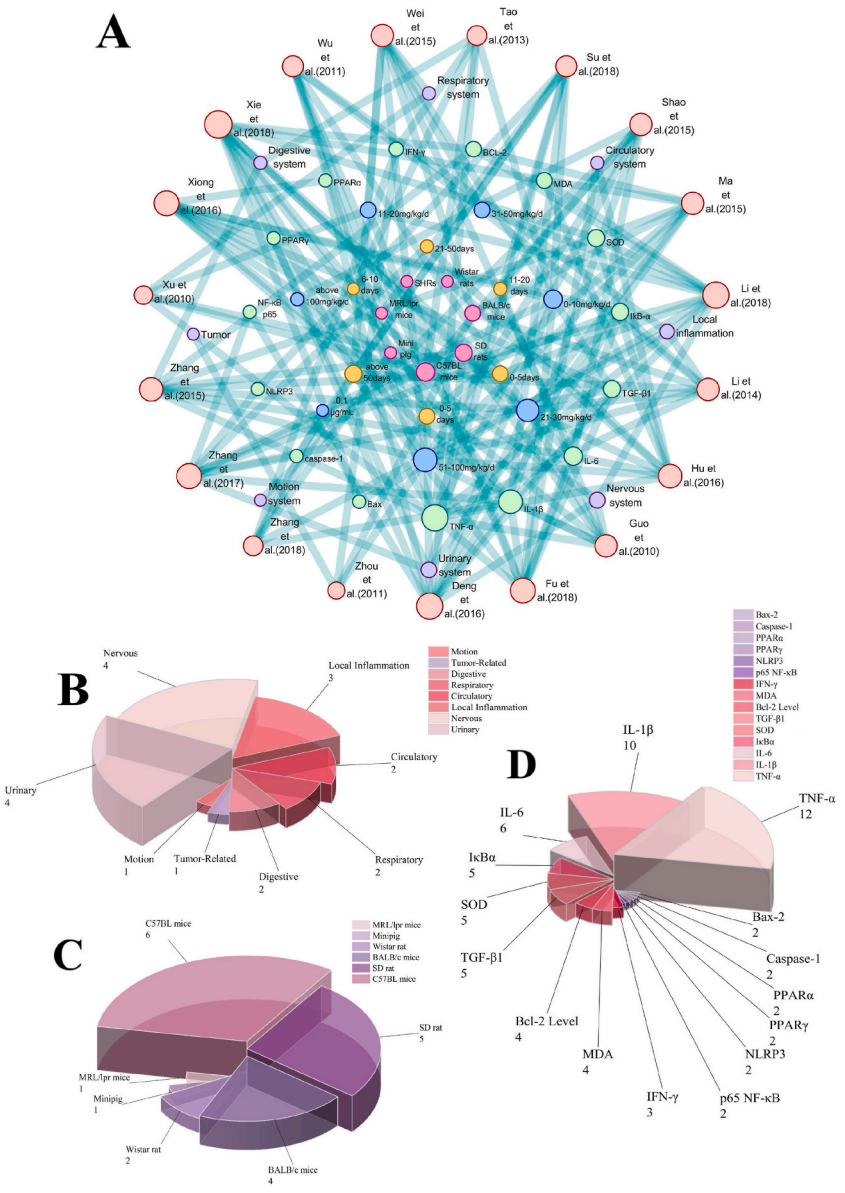


Fig. 3. The basic characteristics of the included literature (A) Include literature network feature map (1. The variables represented by the network feature map from outer to inner are as follows: study, type of disease, outcome measures, dosage, duration of treatment, and species of experimental animals. 2. The size of the point represents the number of connected nodes, the greater the number of connected nodes, the larger the point.). (B) Distribution of disease types. (C) Animal species distribution. (D) Distribution of the number of included outcome indicators.

3.3.1.2. IL-1β. In the analysis of 10 studies, icariin and its derivatives significantly decreased IL-1β levels [$n = 204$, $p = 0.000$, *SMD* = -5.698 (−7.307, −4.089)] (Fig. 5B). Subgroup analysis was necessary to determine the origins of the notable heterogeneity ($I^2 = 84.1$ %) necessitated subgroup analysis to identify the sources of heterogeneity. The "mice" subgroup showed a marked improvement in heterogeneity within the species analysis. According to course, heterogeneity also considerably reduced in the "0–5 days" category. These findings highlight the critical role of species and treatment duration as key heterogeneity factors in IL-1β meta-analysis. (Table 4).

3.3.2. Secondary inflammatory markers

Secondary inflammatory markers demonstrated significant improvements following the administration of Icariin and its analogs (Table 5). They effectively reduced IFN-γ ($p = 0.031$), IL-6 ($p = 0.000$), and TGF-β1 ($p = 0.000$) levels. Additionally, these substances notably elevated IκB-α ($p = 0.000$), NF-κB p65 ($p = 0.000$), and NLRP3 ($p = 0.000$) levels, indicating their anti-inflammatory properties.

Table 2
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((1 mg/kg)	Gavage, ICT, 25/50/100 mg/kg/d, 3 days	Gavage, H_2O, 3 days	①
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,25/50/100 mg/kg/d, 90 days	No procedures	①⑤
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,30/60 mg/kg/d, 28 days	No mention	①③⑩⑪
Fu et al. (2018)	Myocardial fibrosis	Spontaneously hypertensive rats:SHRs (Male, 10/20, 13 weeks)	Spontaneously hypertensive rats	Gavage, ICS II, 4/8/16 mg/kg/d, 84 days	Gavage, distilled water, 84 days	①②③④⑤
Su et al. (2018)	Lupus nephritis (LN)	MRL/lpr mice (10/10, 10 weeks)	MRL/lpr mice	Gavage, ICA, 10 mg/kg/d, 56 days	Gavage, saline, 56 days	①②⑨⑬
Zhang et al. (2017)	IgA nephropathy	SD rats (Male, 15/15, 6 weeks, 180–200 g)	0.1 % BGG (8 weeks)	Gavage, ICA, 10 mg/kg/d, 42 days	Gavage, saline, 42 days	②④⑤⑧⑨⑬
Ma et al. (2015)	Acute renal injury	BALB/c mice (Male, 10/10, 20–22 g)	Cisplatin (15 mg/kg)	Gavage, ICA, 30/60 mg/kg/d, 5 days	Gavage, saline, 5 days	⑩⑪⑭
Guo et al. (2010)	Brain dysfunction	SD rats (Male, 14/14, 10–14 weeks, 250–300 g)	LPS (10 μl)	Gavage, ICA, 30/60/120 mg/kg/d, 17 days	Gavage, distilled water, 17 days	①②
Xiong et al. (2016)	Cerebral I/R	SD rats (Male, 5/5, 16 weeks, 250–280 g)	Reperfusion after MACO (24h)	Gavage, ICA, 10/30 mg/kg/d, 3 days	Gavage, saline, 3 days	②④⑤⑥⑦
Deng et al. (2016)	Cerebral I/R	SD rats (Male, 5/5, 250–280 g)	Reperfusion after MACO (24h)	Gavage, ICS II, 10/30 mg/kg/d, 3 days	Gavage, saline, 3 days	②④⑤⑥⑦⑧
Li et al. (2018)	SCI	C57BL mice (Male, 6/6, 8 weeks)	Spinal cord after laminectomy	Gavage, ICA, 13.53/33.84 mg/kg/d, 3 days	Gavage, saline, 3 days	①②⑩⑪⑭⑮
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, 20 mg/kg/d, 0.25 days	Intraperitoneal injection, PBS (Phosphate Buffered Saline), 0.25 days	①
Wei et al. (2015)	Asthma	BALB/c mice (Female, 20/20,12–16 g)	3 % OVA ovalbumin (21 days)	Gavage, ICA, 20/50/100 mg/kg/d, 56 days	Gavage, saline, 56 days	③④
Xie et al. (2018)	Acute kidney injury	C57BL mice (Male, 10/10, 38–44 weeks)	cecal ligation and perforation (CLP)	Intraperitoneal injection, ICA, 30/60 mg/kg/d, 3 days	No mention	①②③⑩⑪⑭⑮
Zhang et al. (2015)	Intestinal I/R	SD rats (Male, 8/8, 180–220 g)	ischemiaereperfusion (I/R)	Gavage, ICA, 30/60 mg/kg/d, 3 days	Gavage, 0.1 % DMSO, 3 days	①③⑩⑭
Shao et al. (2015)	Osteolysis	C57BL mice (Male, 21/21, 7–8 weeks)	20 mg Ti particle	Gavage, ICA,100/300 mg/kg/d, 14 days	Gavage, saline, 14 days	①②③
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,3/10 mg/kg/d, 10 days,	Gavage, drinking water, 10 days	①⑫
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	②⑫
Zhou et al. (2011)	Tumor	BALB/c mice (Female, 5/5, 6–8 weeks)	Tumor inoculation	Intraperitoneal injection, ICA, 100 mg/kg/d	No mention	⑫

Note: ①TNF-α ②IL-1β ③IL-6 ④TGF-β1 ⑤IkB-α ⑥PPARα ⑦PPARγ ⑧NF-κB p65 ⑨NLRP3 ⑩SOD ⑪MDA ⑫IFN-γ ⑬caspase-1 ⑭BCL-2 ⑮Bax.

3.3.3. Anti-inflammatory/transcriptional regulators

Icariin exhibited a beneficial impact on anti-inflammatory/transcriptional regulators (Table 5), Notably, it led to a significant elevation in PPARα level ($p = 0.000$), This indicator exhibited low heterogeneity contributing to result accuracy.

3.3.4. Anti-oxidative stress indicators

Icariin and its derivatives exhibited notable efficacy as antioxidative stress indicators (Table 5), including significant upregulation of SOD levels ($p = 0.000$) with low heterogeneity ($I^2 = 0.0$ %). Additionally, these compounds inhibited MDA activity ($p = 0.000$),

	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.

Fig. 4. Risk of bias of included studies.

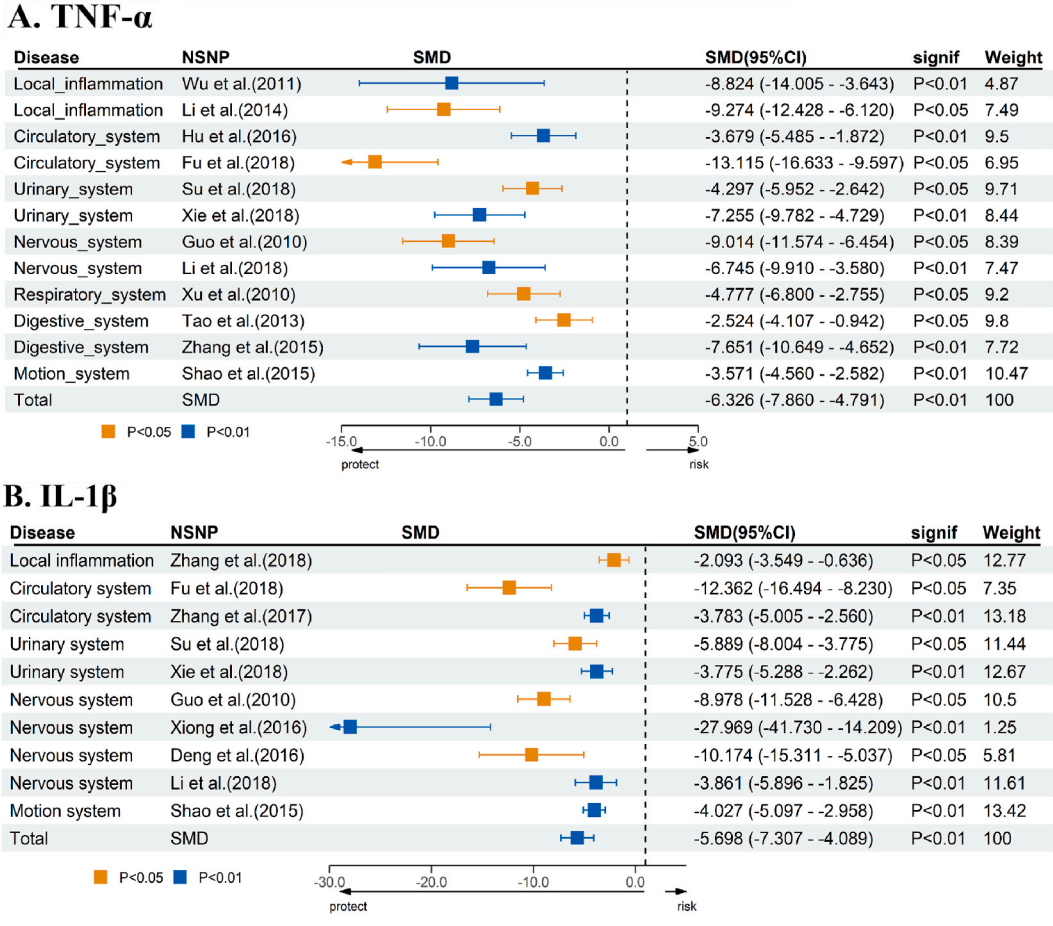


Fig. 5. Effects of Icariin on inhibiting the TNF-α, IL-1β (A)TNF-α. (B)IL-1β.

Table 3
Results of TNF-α subgroup analysis.

Outcome parameters	Individuals (n)	SMD (95%CI)	P-value	Heterogeneit (I^2)
(1) Subgroup analysis based on dose intervals				
0–10 mg/kg/day	$n = 32$	−3.393 (−5.130, −1.656)	$p = 0.000$	$I^2 = 56.6$ %
11–20 mg/kg/day	$n = 36$	−8.817 (−16.983, −0.650)	$p = 0.034$	$I^2 = 93.8$ %
30–50 mg/kg/day	$n = 12$	−6.745 (−9.910, −3.580)	$p = 0.000$	–
51–100 mg/kg/day	$n = 78$	−7.013 (−9.389, −4.636)	$p = 0.000$	$I^2 = 70.8$ %
above 100 mg/kg/day	$n = 70$	−6.159 (−11.487, −0.832)	$p = 0.023$	$I^2 = 93.4$ %
(2) Subgroup analysis based on animal species				
Rats	$n = 88$	−8.198 (−12.134, −4.262)	$p = 0.000$	$I^2 = 88.9$ %
Mice	$n = 150$	−5.314 (−6.765, −3.864)	$p = 0.000$	$I^2 = 74.2$ %
(3) Subgroup analysis based on drug duration				
0–5 days	$n = 72$	−6.471 (−7.806, −5.136)	$p = 0.000$	$I^2 = 10.5$ %
5–10 days	$n = 12$	−2.524 (−4.107, −0.942)	$p = 0.002$	–
11–20 days	$n = 70$	−6.159 (−11.487, −0.832)	$p = 0.023$	$I^2 = 93.4$ %
20–50days	$n = 14$	−3.679 (−5.485, −1.872)	$p = 0.000$	–
above 50 days	$n = 70$	−8.725 (−14.100, −3.350)	$p = 0.001$	$I^2 = 91.4$ %
(4) Subgroup analysis based on sex				
Male	$n = 198$	−6.968 (−8.891, −5.044)	$p = 0.000$	$I^2 = 85.1$ %
Female	$n = 20$	−5.171 (−11.265, 0.923)	$p = 0.096$	$I^2 = 80.7$ %
NA	$n = 20$	−4.297 (−5.952, −2.642)	$p = 0.096$	–
(5) Subgroup analysis based on type of diseases				
Local inflammation	$n = 28$	−9.152 (−11.846, −6.458)	$p = 0.884$	$I^2 = 0.0$ %
Circulatory system	$n = 44$	−8.271 (−17.515, −0.973)	$p = 0.000$	$I^2 = 95.4$ %
Digestive system	$n = 28$	−4.923 (−9.936, −0.091)	$p = 0.003$	$I^2 = 88.6$ %
Urinary system	$n = 40$	−5.616 (−8.498, −2.733)	$p = 0.055$	$I^2 = 72.9$ %
Nervous system	$n = 40$	−8.078 (−10.268, −5.889)	$p = 0.275$	$I^2 = 16.2$ %
Respiratory system	$n = 16$	−4.777 (−6.800, −2.755)	$p = 0.000$	–
Motion system	$n = 20$	−3.571 (−4.560, −2.582)	$p = 0.000$	–

thereby achieving an anti-oxidative stress effect. This contributes to the suppression of the inflammatory processes. Further details are provided in the accompanying tables.

3.3.5. Apoptosis modulating regulators

Icariin and its derivatives showed substantial effects on the modulation of apoptotic regulators (Table 5). They significantly increase BCL-2 levels ($P = 0.000$) and reduced caspase-1 activity ($P = 0.000$). Meta-analysis of caspase-1 and icariin indicated minimal

Table 4
Results of IL-1β subgroup analysis.

Outcome parameters	Individuals (n)	SMD (95%CI)	P-value	Heterogeneity (I^2)
(1) Subgroup analysis based on dose intervals				
0–10 mg/kg/day	$n = 50$	−4.652 (−6.685, −2.619)	$p = 0.000$	$I^2 = 65.0$ %
10–20 mg/kg/d	$n = 20$	−12.362 (−16.494, −8.230)	$p = 0.043$	-
21–30 mg/kg/day	$n = 20$	−17.880 (−35.162, −0.598)	$p = 0.043$	$I^2 = 82.3$ %
31–50 mg/kg/day	$n = 12$	−3.861 (−5.896, −1.825)	$p = 0.000$	-
51–100 mg/kg/day	$n = 20$	−3.775 (−5.288, −2.262)	$p = 0.000$	-
above 100 mg/kg/day	$n = 70$	−6.362 (−11.205, −1.518)	$p = 0.010$	$I^2 = 91.9$ %
NA	$n = 12$	−2.093 (-3.549, −0.636)	$p = 0.005$	–
(2) Subgroup analysis based on animal species				
Rats	$n = 98$	−10.156 (−14.869, −5.443)	$p = 0.000$	$I^2 = 89.7$ %
Mice	$n = 94$	−4.177 (−4.928, −3.427)	$p = 0.000$	$I^2 = 0$ %
(3) Subgroup analysis based on duration of treatment				
0–5 days	$n = 52$	−6.793 (−10.595, −2.992)	$p = 0.000$	$I^2 = 10.5$ %
11–20 days	$n = 70$	−6.362 (−11.205, −1.518)	$p = 0.010$	$I^2 = 91.9$ %
20–50days	$n = 30$	−3.783 (−5.005, −2.560)	$p = 0.000$	–
above 50 days	$n = 40$	−8.872 (−15.195, −2.549)	$p = 0.006$	$I^2 = 86.6$ %
NA	$n = 12$	−2.093 (−3.549, −0.636)	$p = 0.005$	
(4) Subgroup analysis based on sex				
Male	$n = 172$	−6.354 (−8.283, −4.426)	$p = 0.000$	$I^2 = 84.4$ %
Female	$n = 12$	−2.093 (−3.549, −0.636)	$p = 0.005$	–
NA	$n = 20$	−5.889 (−8.283, −4.426)	$p = 0.000$	–
(5) Subgroup analysis based on type of diseases				
Local inflammation	$n = 12$	−2.093 (−3.549, −0.636)	$p = 0.005$	–
Circulatory system	$n = 50$	−7.836 (−16.230,0.559)	$p = 0.067$	$I^2 = 93.4$ %
Urinary system	$n = 40$	−4.698 (−6.753, −2.643)	$p = 0.000$	$I^2 = 60.6$ %
Nervous system	$n = 60$	−9.456 (−14.526, −4.386)	$p = 0.000$	$I^2 = 86$ %
Motion system	$n = 42$	−4.027 (−5.097, −2.958)	$p = 0.000$	–

heterogeneity ($I^2 = 7.9$ %).

3.3.6. Publication bias

The evaluation for publication bias in the meta-analysis focused on primary markers. The Egger's test for TNF-α showed a t-value of −4.59 (Fig. 6A), with the t-value's absolute exceeding 0.05, indicating no publication bias. For IL-1β, Egger's test reported a t-value of −4.21 (Fig. 6B), also exceeding the 0.05 threshold, suggesting an absence of publication bias.

3.4. Machine learning

3.4.1. Characteristics of the study sample and correlation analysis

The analysis included 260 samples that were treated with icariin and its derivatives at doses ranging from 3 to 300 mg/kg/day over 0.25–90 days. The distribution of the time-dose efficacy is depicted as follows (Fig. 7). For categorical variables, Kendall's method provided a correlation analysis (Fig. 8A), whereas Pearson's method was applied to continuous variables (Fig. 8B). All absolute correlation coefficients were below 0.35, indicating a low likelihood of collinearity.

3.4.2. Model construction and assessment

Four distinct machine learning techniques were employed to develop individual base models, which were then integrated into a metamodel. The Stacking algorithm facilitates the integration of features within the metamodels. Following hyperparameter optimization, all four meta-models achieved *RMSE* values below 0.32, indicating strong predictive capability. The basic models, established using the same machine-learning algorithms and fitted with the stacking algorithm, yielded good prediction performance, with *RMSE* values below 0.43 (Fig. 9A). Subsequently, these models underwent regularization, resulting in an optimal performance when the penalty parameter was set at 0.0173 and the mixture value was set at 1. Under these conditions, the meta-model achieved an *RMSE* of 0.208 and R^2 of 0.954, demonstrating effective prediction (Fig. 9B). Moreover, the Stacking model exhibited favorable performance in both the training and test sets, with *RMSE* of 0.193 and 0.289, R^2 of 0.962 and 0.928, and *MAE* of 0.068 and 0.116, respectively, confirming its reliability (Fig. 9C and D).

3.4.3. Interpretation of the models

The prediction model's SHAP value variable significance map (Fig. 9A) was created, with five significant features prioritized according to how they affected TNF-α levels. Notably, "Type of disease," "Animal species," and "Dose" exhibited significant contributions to TNF-α level improvement (Fig. 10A). Darker hues on the SHAP value summary chart imply greater efficacy, and higher SHAP values for a characteristic suggest a larger chance of increasing TNF-α efficacy. In terms of categorical variables, icariin and its derivatives were particularly effective in improving TNF-α efficacy across respiratory, urinary, neurological, and digestive disease types. Moreover, "Wister mice" and "BALB/c mice" species, along with "female" sex, contributed favorably to efficacy improvement. Regarding continuous variables, icariin and its derivatives showed enhanced efficacy when the treatment duration exceeded 31.22 days or when the dose surpassed 27.52 mg/kg/day (Fig. 10B and C).

Table 5
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*)	Individuals (*n*)	*SMD 95 % (CI)*	*P-value*	Heterogeneity (I^2, *P-value*)
(1) Secondary indicators of inflammation					
IL-6	$n = 6$	$n = 162$	−6.419 (−9.101, −3.738)	$p = 0.000$	$I^2 = 94.4$ %, $p = 0.000$
IFN-γ	$n = 3$	$n = 34$	−2.173 (−4.146, −0.200)	$p = 0.031$	$I^2 = 77.9$ %, $p = 0.011$
TGF-β1	$n = 5$	$n = 120$	−6.212 (−8.363, −4062)	$p = 0.000$	$I^2 = 80.4$ %, $p = 0.000$
IkB-α	$n = 5$	$n = 100$	16.793 (8.302, 25.284)	$p = 0.000$	$I^2 = 90.6$ %, $p = 0.000$
NF-κB p65	$n = 2$	$n = 40$	3.830 (2.747,4.914)	$p = 0.000$	$I^2 = 0.0$ %, $p = 0.425$
NLRP3	$n = 2$	$n = 50$	5.822 (2.921,8.723)	$p = 0.000$	$I^2 = 0.0$ %, $p = 0.047$
(2) Anti-inflammatory/transcriptional regulators					
PPARα	$n = 2$	$n = 20$	3.129 (1.674,4.584)	$p = 0.000$	$I^2 = 4.7$ %, $p = 0.047$
PPARγ	$n = 2$	$n = 20$	3.220 (−0.429,6.868)	$p = 0.084$	$I^2 = 80.7$ %, $p = 0.023$
(3) Anti-oxidative stress indicators					
SOD	$n = 5$	$n = 82$	3.623 (1.871,5.375)	$p = 0.000$	$I^2 = 0.0$ %, $p = 0.047$
MDA	$n = 4$	$n = 66$	−6.695 (−9.167, −3.773)	$p = 0.000$	$I^2 = 82.5$ %, $p = 0.000$
(4) Apoptosis regulators					
BCL-2	$n = 4$	$n = 68$	10.439 (4.977,15.901)	$p = 0.000$	$I^2 = 90.1$ %, $p = 0.000$
caspase-1	$n = 2$	$n = 50$	−6.474 (−7.995, −4.953)	$p = 0.000$	$I^2 = 7.9$ %, $p = 0.297$
Bax	$n = 2$	$n = 32$	−14.153 (−40.657, 12.351)	$p = 0.295$	$I^2 = 94.4$ %, $p = 0.000$

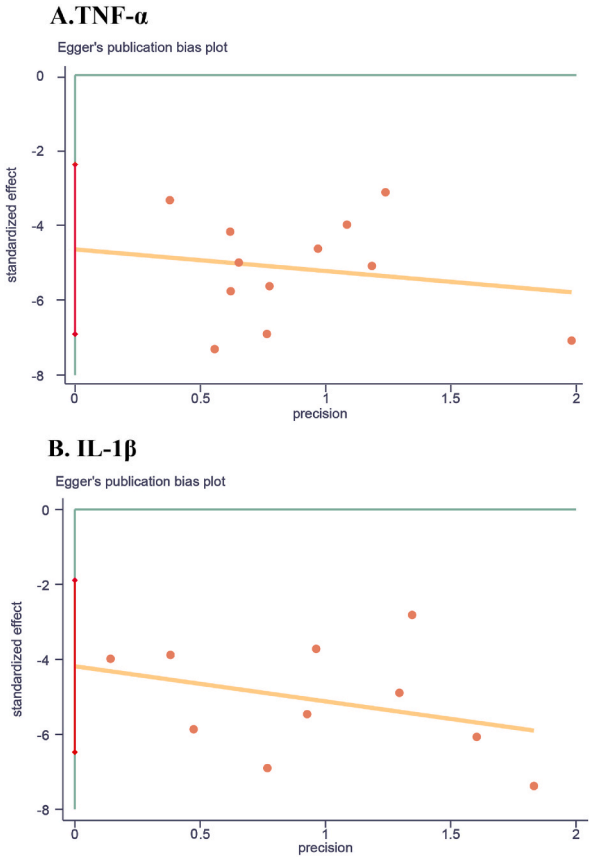
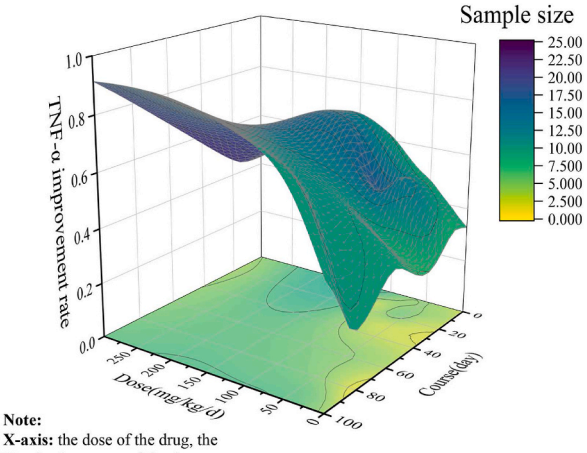


Fig. 6. Publication bias based on TNF-α and IL-1β (A) TNF-α. (B) IL-1β.



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)

Fig. 7. Time-dose-efficacy interval distribution with TNF-α as curative effect standard.

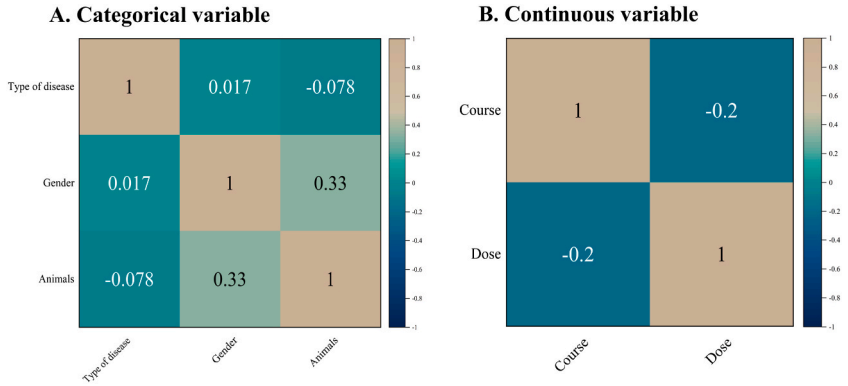


Fig. 8. Correlation Between Independent Variables (A) Correlation Between Categorical Independent Variables. (B) Correlation between continuous independent variables.

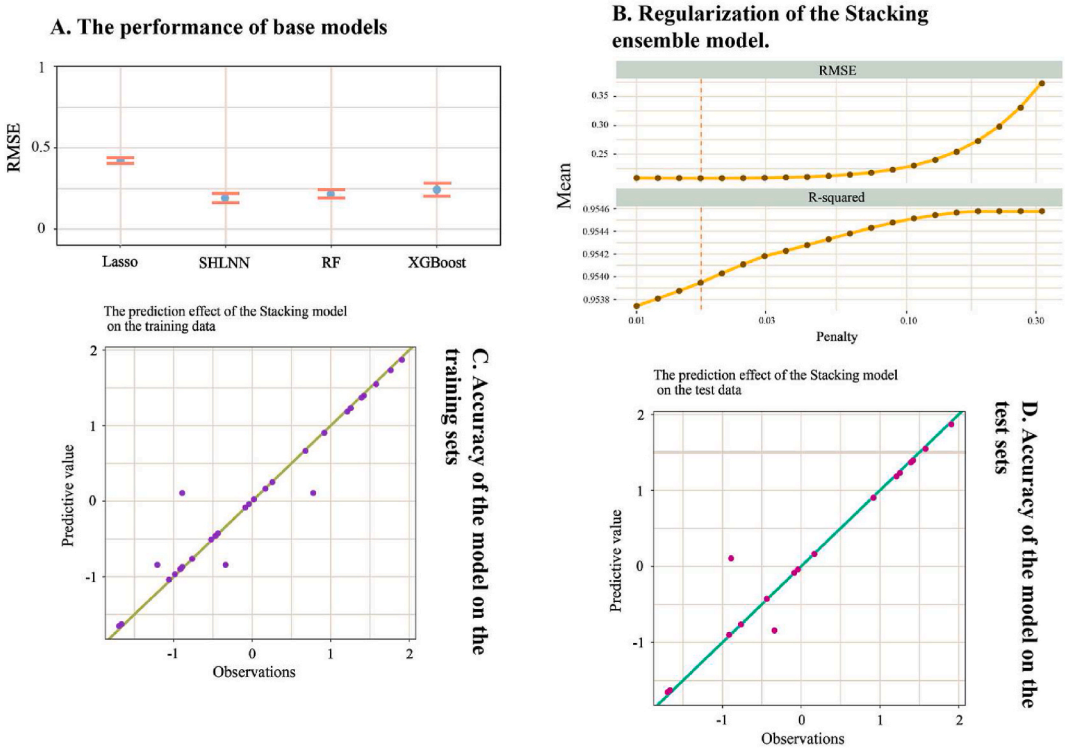


Fig. 9. The performance of base models, regularization of the Stacking ensemble model, and the accuracy of the model on the dataset (A) The performance of base models. (B) Regularization of the Stacking ensemble model. (C) Accuracy of the model on the dataset.

4. Discussion

4.1. Synthesis of the evidence

In order to determine the effectiveness of icariin and its derivatives in managing inflammation, this study performed both a machine-learning analysis on a sample dataset and a primary meta-analysis incorporating 19 studies, totaling 370 animals in the meta-analysis and 260 animals in the machine-learning techniques. These findings revealed the therapeutic potential of icariin and its derivatives in various diseases by modulating cytokine levels through related pathways.

4.2. Potential mechanism

Multifaceted in nature, inflammation is a key factor in the initiation and development of many illnesses, such as cancer [42], acute

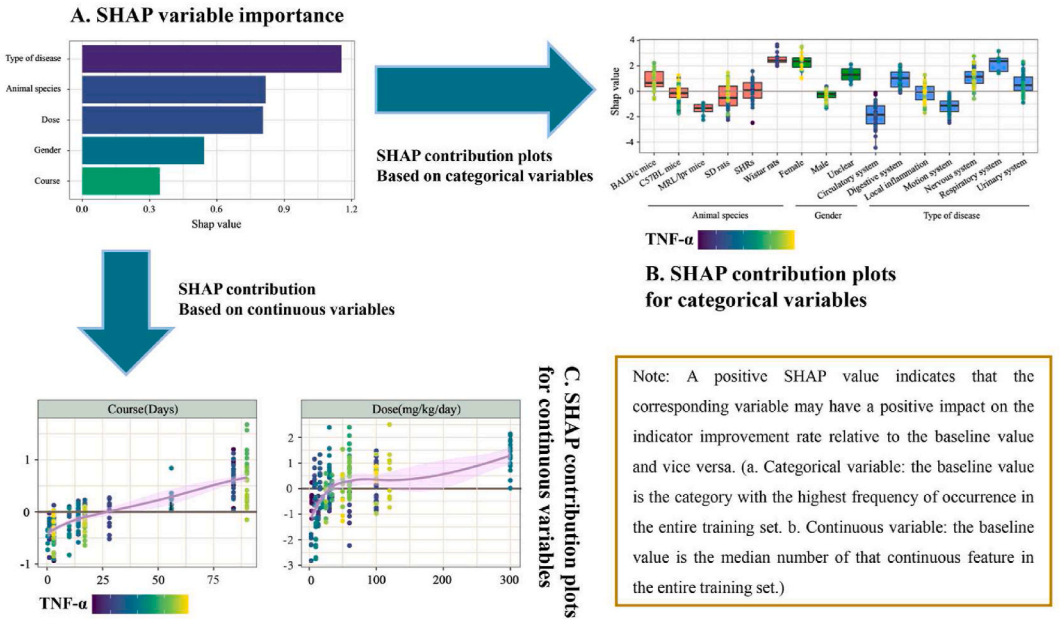


Fig. 10. SHAP variable importance and SHAP contribution plots based on variable types (A) SHAP value variable importance map. (B) SHAP contribution plot based on categorical variables. (C) SHAP contribution plot based on continuous variable.

kidney injury [43], stroke [44], AS [45]etc. This complex response involves multiple cell types, cytokines, and pathways. Infiltration of immune cells, particularly by macrophages and neutrophils, is a crucial step in the inflammatory cascade. Upon activation, immune cells release cytokines such as IL-1 and TNF-α, which further stimulate T and B cell activation, proliferation, and differentiation [46]. Macrophages also secrete chemokines that attract monocytes to the sites of inflammation (Fig. 11).

Studies have strongly linked inflammation to atherosclerosis (AS) development, particularly highlighting the role of foam cell formation within arterial walls due to macrophages engulfing cholesterol [47,48]. Class B Scavenger Receptor Type I (SR-BI), a protein critical for Lipoprotein, High-Density (HDL) transport [49], aids in HDL uptake and cholesterol efflux, thus preventing foam cell creation [50,51]. Icariin enhances SR-BI expression, facilitates HDL uptake and cholesterol efflux, and reduces foam cell formation, alleviating AS [52]. Additionally, Cluster of Differentiation 36 (CD36), a receptor involved in cholesterol transport and low-density lipoprotein oxidation, contributes to AS development and progression [53–55]. The P38 MAPK pathway activates CD36 and is involved in various diseases [56]. Icariin derivatives inhibit this pathway by decreasing CD36 activity and slowing AS progression [52]. In myocarditis, the NF-κB pathway is pivotal in disease pathogenesis [57]. Myocardial inflammation is decreased by Sirtuin 6 (SIRT6), which downregulates NF-κB and lowers inflammatory markers such as TNF-α and ICAM-1 [58,59]. Icariin increases SIRT6, inhibiting NF-κB and consequently reducing inflammation [60]. The JNK pathway plays a crucial in inflammation [61]. JNK's principal transcription factor, c-Jun, has been connected to inflammation and apoptosis [62]. The fact that NF-κB may increase c-Jun's activity and exacerbate inflammation is crucial to understand [63]. However, Zhou et al. discovered that icariin can mitigate inflammation by curtailing the activity of c-Jun, achieved via preventing the activation and release of NF-κB [64]. TGF-β activates Samd2 phosphorylation, promoting cardiac fibroblast transformation and collagen synthesis [65]. Icariin and its derivatives effectively reduce TGF-β levels and impede Samd2 activity, inhibiting collagen synthesis. Interestingly, ICS II's regulation of IκB phosphorylation also suppresses NF-κB's nuclear translocation and expression [30], aiding myocardial inflammation's treatment.

Inflammation is pivotal in urological conditions, with the NLRP3 inflammasome, regulated by NF-κB, playing a crucial role in modulating inflammatory responses [66–68]. Icariin lowers NLRP3 levels by suppressing NF-κB, thereby alleviating inflammation in diseases such as lupus nephritis and IgA nephropathy (IgAN) [38,40]. Additionally, the inflammasome PYD and CARD Domain Containing, which facilitates the formation of caspase-1, thereby raising IL-1β levels in IgAN, is inhibited by icariin, thereby impeding this conversion and reducing inflammation [69,70]. IKKβ, a protein kinase, phosphorylates IκBα to activate NF-κB, promoting inflammation [71,72]. Icariin delays this process in IgAN, reduces renal damage, and mitigates IgA deposition and mesangial matrix expansion [38]. In acute kidney injury, icariin suppresses NF-κB, improving levels of TNF-α and inducible Nitric Oxide Synthase 2 (NOS2). This procedure is also useful for treating nephrotoxicity [24,33].

Inflammation is a major contributor to the development of neurological disorders. PPARα and PPARγ act to suppress NF-κB expression in cerebral ischemia-reperfusion injury by preventing the phosphorylation modification of IκBα [73,74]. Icariin derivatives, such as ICS II and IRS, elevate PPARα and PPARγ levels, further inhibiting inflammatory pathways [29,36]. In neurodegenerative diseases, Nrf2 increases SOD levels and reduces reactive oxygen species (ROS), thereby inhibiting MAPK pathways [75–78]. Additionally, icariin enhances Nrf2 levels to reduce inflammation [79]. Furthermore, the Phosphatidylinositol 3-kinase/Protein Kinase B (PI3K/PKB) pathway, which promotes the release of inflammatory factors in nerve injury, is inhibited by icariin, thereby blocking

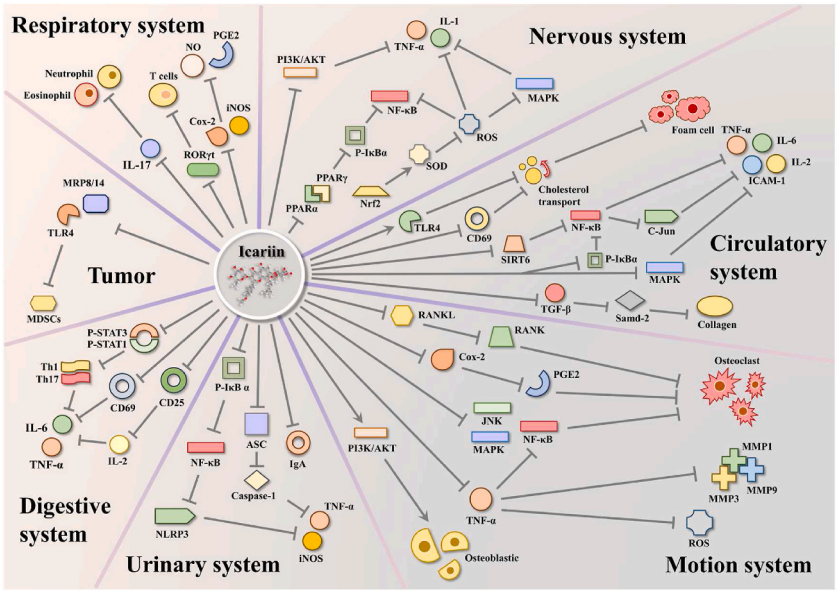


Fig. 11. Icariin's potential anti-inflammatory mechanism.

inflammation [80,81].

In respiratory diseases, inducible NOS2 and Prostaglandin-endoperoxide synthase 2 (Cox-2) play a significant role in inflammation, primarily by promoting Nitric Oxide (NO) and Prostaglandin E2 (PGE2) production in response to bacterial and viral stimuli [82,83]. Icariin decreases the production of these inflammatory mediators by inhibiting the activities of NOS2 and Cox-2 [34]. In asthma, airway inflammation, which is largely driven by neutrophil and eosinophil infiltration, is significantly influenced by Interleukin-17 (IL-17) [84]. The ability of icariin to reduce IL-17 levels plays a crucial role in alleviating airway inflammation [23]. It also limits excessive mucus secretion by the mucous gland cells, thereby further reducing airway inflammation [85]. Forkhead box protein P3 (Foxp3) is a key regulator of Tregs in the immune regulatory T cells (Treg cells). RORγt, a transcription factor, can bind to Foxp3, reducing its activity and thus impacting Treg cell development and function, thereby modulating inflammation [86]. Icariin elevates RORγt levels, diminishing the promotive effect of Foxp3 on Treg cell differentiation [23].

Icariin has shown promise as a multimodal treatment for inflammatory gastrointestinal disorders. When a person has colitis, Signal Transducer and Activator of Transcription (STAT) 3 and 1 encourages these cells to differentiate into Th1 and Th17 cells, which increases the release of inflammatory molecules including IL-17 and IFN-γ [87–90]. Icariin inhibited STAT1 and STAT3 phosphorylation [27]. Additionally, T cell activation and proliferation markers, Cluster of Differentiation 69 and 25 (CD69 and CD25), which respectively increase Interleukin-2 (IL-2) receptor affinity and T cell activity respectively [91,92], enhance the affinity of the IL-2 receptor and T cell activity [93]. Inhibition of CD25 and CD69 by icariin dampens T-cell differentiation and function [27].

Inflammation significantly affects motor system disorders. The interaction between Osteoprotegerin Ligand and Receptor Activator of Nuclear Factor Kappa-B (OPGL-RANK), which is crucial in osteolytic diseases, activates osteoclasts and results in osteolysis [94]. Icariin, a naturally occurring compound, effectively downregulates OPGL and inhibits its binding to RANK, thereby reducing osteolysis [31]. Additionally, the enzyme Cox-2 facilitates the conversion of arachidonic acid to PGE2, which promotes osteoclast differentiation [95]. Icariin counteracts this by inhibiting Cox-2, thereby diminishing osteoclast differentiation [96]. Significantly, icariin also inhibits pathways like NF-κB, P38 MAPK, and JNK, all of which are known to stimulate osteoclast formation and osteolysis [97,98]. Icariin can inhibit this process [96,99]. In degenerative disc diseases, the PI3K/PKB pathway, which aids bone matrix formation, is enhanced by icariin [100]. In Osteomyelitis and Chondritis scenarios, increased levels of TNF-α play a pivotal role in elevating Matrix Metalloproteinases (MMP1, MMP3, MMP9, and MMP13) and ROS, further activating NF-κB P65 and promoting inflammation [101–103]. However, icariin counteracts this process by suppressing TNF-α levels, thereby reducing inflammation [104–106].

Research has indicated a significant link between chronic inflammation and tumor progression [107]. Myeloid-derived suppressor cells (MDSC) play a crucial role in tumor growth by impeding adaptive immunity. This effect is enhanced by chronic inflammation, particularly through the upregulation of Toll-like receptor 4 (TLR4) and MRP8/14, which augment MDSC activity [108,109]. Notably, compounds, such as ICA and ICT, have been found to decrease TLR4 and MRP8/14 levels, consequently inhibiting MDSC activation and slowing tumor progression [37].

4.3. Implications

As a major pathogenic element of many disorders, inflammation is usually treated with glucocorticoids and non-steroidal anti-inflammatory medications. However, the efficacy of plant monomers as anti-inflammatory agents remains underutilized, partly

because of the gap in translating animal study results into clinical outcomes and the lack of comprehensive preclinical data. This research looked on the possible therapeutic of icariin in treating inflammation by employing a meta-analysis of outcome indices and machine learning to identify optimal dosages and treatment durations. Additionally, we investigated specific inflammatory diseases for which icariin and its derivatives are the most effective. The findings indicate that icariin and its derivatives positively modulate markers like TNF-α, IL-1β, and so on. Notably, these compounds exhibited enhanced anti-inflammatory effects against respiratory diseases and similar conditions, especially with dosages above 27.52 mg/kg/day and treatment durations exceeding 31.22 days.

Animal experimentation, which is a fundamental aspect of medical research, consistently faces ethical considerations [110]. The increasing number and scale of these experiments have led to stricter ethical reviews regarding their conduct [111]. Consequently, the efficient selection of animal models, treatment plans, and dosage levels, as well as the effective utilization of results have become critical [112]. Animal meta-analyses address this issue by integrating existing experimental data, conserving resources, and providing robust and precise preclinical evidence. Additionally, machine learning assists in identifying key features for disease treatment and offers valuable insights for refining future animal experiments. Research has revealed that although numerous drugs exhibit promising therapeutic effects in laboratory settings, the rate of successful clinical application remains low [113]. This disparity partly stems from anatomical and physiological differences between animal models and humans, despite the shared mechanisms identified in laboratory studies. For the purpose of closing this gap and speeding up the clinical translation process, machine learning can be used to extract important data and choose the best animal model. Based on this, the subsequent steps will entail conducting more targeted animal studies, informed by the conclusions of the systematic review and the interpretative results of the machine learning models. Concurrently, drug toxicity assessments will be undertaken to further evaluate the safety profile of icariin, complemented by studies on its pharmacokinetic properties. By synthesizing insights derived from machine learning and systematic reviews, we can enhance the selection process of animal models, refine treatment protocols, and ascertain optimal dosage levels. Ultimately, this strategy seeks to bridge the gap between laboratory research and clinical application, thereby improving the efficacy and applicability of icariin as a therapeutic agent.

4.4. Limitations

(1) This study encompasses 19 English-language studies with baseline feature descriptions provided only by Li et al., Su et al., Guo et al., Xu et al., Tao et al., and Wei et al. Performance-biased blinding was not described in Deng et al., Shao et al., and Xu et al., and the majority of studies, including Hu et al., reported incomplete outcome data. (2) Heterogeneity was observed in the meta-analysis. Despite improvements in some subgroups through subgroup analysis, other subgroups continued to exhibit heterogeneity influenced by diverse factors such as experimental practices, laboratory environments, and geographical variables. (3) The machine-learning model demonstrated high interpretability; however, the scarcity of sample data could potentially affect the feature screening accuracy. (4) Among the four models selected in this study, XGBoost and RF are prone to overfitting with limited data, whereas Lasso is sensitive to the selection of regularization parameters. Furthermore, SHLNN exhibits limited adaptability to complex data structures. While the integration of these models through the Stacking algorithm can alleviate some of the individual model limitations, it also increases the overall complexity of the model and poses challenges for interpretability.

5. Conclusion

Icariin exhibited significant efficacy against diseases driven by inflammation, especially respiratory, neurological, urinary, and digestive system disorders. The anti-inflammatory benefits were particularly pronounced at doses above 27.52 mg/kg/day and for treatments extending beyond 31.22 days. These compounds influence key signaling pathways, such as PI3K/PKB, across various inflammatory scenarios. Moving forward, more targeted animal studies and safety assessments will be conducted to obtain more comprehensive preclinical evidence to support the potential of icariin's promise as a clinical treatment agent for inflammatory illnesses.

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Data availability statement

Although they have not been placed in a publicly accessible repository, the study's data are accessible upon justifiable request. To negotiate terms of access, researchers who are interested in using the data can get in touch with the corresponding author.

CRediT authorship contribution statement

Xiaochuan Guo: Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Yanqin Qin:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation. **Zhenzhen Feng:** Software, Project administration, Methodology, Funding acquisition. **Haibo**

Li: Software, Formal analysis, Conceptualization. **Jingfan Yang:** Software, Formal analysis. **Kailin Su:** Software. **Ruixiao Mao:** Software. **Jiansheng Li:** Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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