• Review Article •

Efficacy and mechanism of flavonoids in improving dry eye disease: a systematic review of animal studies

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Abstract

● **AIM:** To systematically summarize the efficacy and safety of flavonoids in the treatment of dry eye disease (DED), and review their mechanisms of action, and provide a scientific foundation for clinical translation.

● **METHODS:** To retrieve papers published from the establishment of the database through May 12, 2025, eight databases were searched: the Chinese National Knowledge Infrastructure (CNKI), the China Biomedical Literature Database (CBM), the Wanfang Database, the China Science, Technology Journal Database, PubMed, the Cochrane Library, Embase, and the Web of Science. Two independent researchers conducted the literature selection and information extraction processes, utilizing the SYRCLE tool to assess the risk of bias. The results of eligible studies were subjected to narrative analysis.

● **RESULTS:** A total of 11 animal studies were included in this review, encompassing research conducted in China, the United States, Republic of Korea, and Japan. These studies involved six flavonoid-rich substances, such as *Buddleja officinalis* and purple corn extract (PCE), as well as four individual flavonoids including quercetin and daidzin, *etc*. The findings indicated that flavonoids have the potential to enhance tear secretion. Following interventions with quercetin eye drops and PCE administered at varying doses and time intervals, a significant increase in tear secretion was observed, which approached normal levels. Additionally, these interventions demonstrated a capacity to mitigate damage to the corneal epithelium. For instance, maqui berry extract (MBE) were found to improve corneal fluorescein staining scores while reducing damage to the corneal surface. Flavonoids significantly alleviate dry eye symptoms in animal models by enhancing tear secretion and mitigating corneal epithelial damage. The observed efficacy is attributed to a range of mechanisms, including anti-inflammatory, antioxidant, hormonal regulation, and anti-apoptotic effects.

● **CONCLUSION:** The consistent therapeutic outcomes noted in both mouse and rat models further underscore the translational potential of these compounds. Consequently, flavonoids are regarded as highly promising natural agents for ocular health. However, additional pharmacokinetic studies and clinical trials are necessary to confirm their efficacy and safety in human subjects with dry eye syndrome.

● **KEYWORDS:** flavonoids; dry eye disease; systematic review; animal studies

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INTRODUCTION

Dry eye disease (DED) is a multifactorial, chronic ocular surface disorder characterized by abnormalities in tear quality or quantity, as well as an imbalance within the ocular surface microenvironment. DED is characterized by the disruption of tear film homeostasis, which can result in ocular discomfort, visual impairment, and potential damage to the ocular surface tissues[1]. The clinical manifestations of DED exhibit considerable heterogeneity; patients often report subjective symptoms, including sensations of dryness in the eyes, a foreign body sensation, burning sensations, and asthenopia. Objective signs may encompass conjunctival hyperemia, a shortened tear film break-up time, positive corneal fluorescein staining characterized by dot patterns, and in more severe cases, filamentous keratitis[2]. Epidemiological data suggest a global prevalence that varies between 5% and 50%, with notably elevated rates observed in older populations and among women[3]. The prevalence of DED has been steadily increasing each year, closely linked to contemporary lifestyle

factors and environmental influences. These include prolonged use of digital screens, the wearing of contact lenses, exposure to air pollution, and living in low humidity environments[4].

The etiology of DED is complex and multifactorial, involving tear film instability, hypertonicity of tears, inflammation and damage to the ocular surface, as well as abnormalities in sensory nerve function[5]. Among these factors, chronic inflammation represents the primary pathological mechanism that drives disease progression. Recent studies have clarified that the cascade of inflammatory mediators—such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)—in both the lacrimal gland and ocular surface epithelium plays a crucial role in advancing the condition[6]. Currently, the primary approach for managing DED primarily involves the use of artificial tears to relieve symptoms[7]. However, this approach offers only temporary alleviation of discomfort and focuses on symptomatic relief rather than addressing the underlying causes. Consequently, this presents challenges in reversing the inflammatory process and fundamentally restoring glandular function[8-9].

Flavonoids, as a large group of polyphenols that are abundantly found in plants, fruits, vegetables, and tea, exhibit significant anti-inflammatory and antioxidant properties, along with various other pharmacological activities[10-11]. According to the differences in their core structures, flavonoids can be categorized into seven distinct groups: flavonoid, flavonol, flavanone, isoflavone, chalcone, flavanolone, and anthocyanidin[12]. Given the chronic inflammatory characteristics of DED and the limitations associated with current treatment options, nutritional intervention has garnered interest as a potential safe strategy. Research has demonstrated that flavonoids may mitigate symptoms associated with DED by modulating immune responses, decreasing oxidative stress, and inhibiting inflammatory processes[13-14]. Furthermore, several studies indicate that its role in regulating intestinal flora and metabolites may also play an indirect part in immune regulation[15]. However, the findings from recent studies regarding the impact of flavonoids on alleviating DED are not entirely consistent[16-17]. The discrepancies observed may arise from various factors, including the use of different types of flavonoids in studies, variations in animal models employed, diverse administration methods, and differing dosage regimens. Additionally, there may be inconsistencies in the core indicators utilized for evaluating efficacy. Currently, there remains a significant gap in the systematic summarization, quality control, quantitative comparison of effect sizes, and comprehensive organization of the disparate animal research evidence. Consequently, this study aims to conduct a systematic review of existing animal studies to elucidate the actual effects of flavonoids on improving the core symptoms and signs of DED across various animal models. This endeavor seeks to provide a scientific foundation for future clinical translation and application.

MATERIALS AND METHODS

The protocol for this systematic review is registered on PROSPERO (CRD420250655978). We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist[18] and its PRISMA for Abstracts checklist[19] for the conduct and reporting of this review.

Inclusion Criteria Subjects: experimental rats, regardless of type, male or female; intervention measures: the mouse and rat were treated with flavonoid rich substances or flavonoids; the control group was model blank control, vehicle control or conventional treatment; outcome measures: tear secretion, corneal epithelial damage; study type: randomized controlled animal studies, language limited to English and Chinese.

Exclusion Criteria Interventions combined with other drugs or active substances; studies without full text available; duplicate published studies; research proposals, conference papers, abstracts, *etc*.

Search Strategy The search was independently conducted by two researchers and systematically covered eight databases, namely, Chinese National Knowledge Infrastructure (CNKI), the Wanfang Database, the China Biomedical Literature Database (CBM), China Science and Technology Journal Database, PubMed, the Cochrane Library, Embase, and the Web of Science. We employed a combination of subject headings and free-text terms, with the search period spanning from each database's inception to May 12, 2025. English search terms are: xerophthalmia, dry eye, dry eye disease, DED, flavonoid*, flavone*, flavonol*, flavanone*, isoflavone*, chalcone*, flavan*, anthocyanidin*, anthocyanin*, polyphenol*, plant extract, phenolic, benzopyrone, flavanonol*, naringenin, quercetin, eupatilin, luteolin, isorhamnetin, genistein, daidzin, xanthohumol, catechin, hesperidin, apigenin, kaempferol, animal model, murine, mouse, rat.

Literature Screening and Data Extraction By two independent literature researchers in related training selection and data extraction, in case of disagreements are resolved through discussion with a third person. Literature into Endnote X9 document management software, to eliminate repetition has nothing to do with literature, reading in this paper, and the full text, according to include and exclude standard screening literature and extract the relevant information. Extract the information of basic information, including research published as the first author's last name, year, study site, groups of sample size, means of intervention, the intervention time, ending index, *etc*.

Risk of Bias Assessment Those evaluated by the SYRCLE animal experiment risk of bias assessment tool (Center for

Systematic Evaluation of Laboratory Animal Research) were selected[20], mainly including ten items: production of allocation sequence or application; each baseline whether the same or whether to adjust confounding factors; distribution of hidden; whether animals were randomly housed during the experiment; whether animal breeders and researchers were blinded; results whether the animals were randomly selected; whether the outcome evaluators were blinded; whether incomplete data are reported; whether study reporting is irrelevant to selective outcome reporting; whether there is no other bias. Determine the results are divided into: low risk of bias, high risk of bias and uncertain risk of bias.

Methods of Data Analysis In this review, a narrative analysis of the eligible outcomes was performed utilizing text and tables that focused on the participants, the substance of the intervention, and its impact on the outcome indicators.

RESULTS

Literature Search Results A total of 1075 relevant studies were initially retrieved from the databases. The deduplication process was performed using Zotero reference management software. Zotero's built-in duplicate detection algorithm automatically identifies potential duplicates by comparing key fields such as title, author, journal, and DOI. Following automatic identification, all potential duplicates were manually reviewed to confirm matches and ensure accuracy, resulting in the removal of 532 duplicate records and resulting in 543 records. After reviewing the titles and abstracts, 491 publications were excluded for not meeting the inclusion criteria. Among these, 395 were irrelevant studies, 56 were reviews, and 40 were trial registrations. Ultimately, 52 potentially eligible studies were identified. Of these, 4 were excluded due to unavailability of the full text, 14 were clinical research studies, 8 had inconsistent outcome measures, and 15 exhibited discrepancies in the intervention or control methods. The literature screening process and results are presented in Figure 1.

Basic Characteristics of Included Literature A total of 11 studies[21-31] were included in this study. Studies from China, the United States, Republic of Korea, and Japan. It incorporates a complex flavonoid component from six plant sources, namely, *Buddleja officinalis*, purple corn extract (PCE), purple sweet potato (PSP), ethanol extract of *R. volubilis* (EERV), *Aster koraiensis* extract (AKE), maquiberry extract (MBE). Four single flavonoids, namely quercetin, epigallocatechin gallate (EGCG), daidzin, acacetin. Specific results are shown in Table 1.

Risk of Bias of the Included Studies Of the 11 included studies, 11 only mentioned randomization. No study reported whether each group was blinded at baseline. Eight articles[21-22,25-26,28-31] reported that experimental animals were housed under the same conditions and environment. No

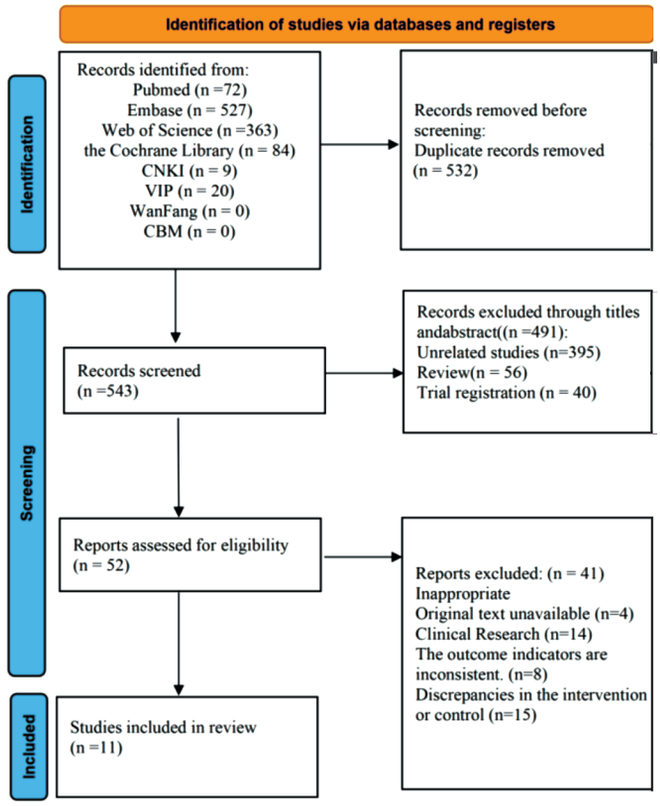

Figure 1 Literature screening process.

studies explicitly reported randomized assessment of outcome measures. Eight articles[21-23,26-30] had complete data reporting. The results of bias risk assessment of the included studies are shown in Figures 2 and 3.

Effects of Flavonoid Rich Substances and Flavonoids on Tear Secretion Five studies[23-24,27,29-30] examined the impact of flavonoid-rich substances on tear secretion. The extract of *Buddleja officinalis* was found to enhance the expression of androgen receptors in lacrimal glands, thereby sustaining basal tear secretion in castrated rats[23]. This effect became evident after one month of intervention and appeared to stabilize with prolonged treatment duration. After the oral administration of PCE at a dosage of 150 mg/kg for one week, the recovery of tear secretion was found to be superior to that observed in the omega-3 control group[24]. This effect is attributed to the inhibition of inflammation and apoptosis within the lacrimal glands. PSP contributes to the maintenance of tear secretion capacity by alleviating oxidative stress induced by a high-fat diet[27]. At the cellular level, PSP administration ameliorates lacrimal gland morphology and attenuates glandular atrophy. Furthermore, it supports myoepithelial cell function, as evidenced by sustained expression of α-smooth muscle actin. PSP also preserves the expression of aquaporin-5, a critical protein involved in tear fluid secretion, thereby underpinning the secretory competence of the lacrimal gland. Furthermore, EERV and AKE, administered at doses of 50 and 100 mg/kg, respectively, demonstrated more favorable effects compared

Table 1 Basic characteristics of the included studies

First author, y	Country	Intervention substance	Flavonoids	Animal species/sex/age	Modeling method	Intervention and groups	Sample size	Frequency	Outcome measurement method	Time of measurement	Ending details	Adverse events
Oh[21], 2015	Republic of Korea	Quercetin	Flavonol	NOD.B10.H2b mice aged 12 to 16wk	Subcutaneous scopolamine injection +low-humidity fan-exposed environment	Three groups: blank group, PBS group, quercetin group	5	Four times a day for 10d	Phenol red thread test; corneal irregularity score; H&E staining; PAS staining	3, 5, 7, 10d	The tear volume after 10d of dry stress was lower than that at baseline; in quercetin group, tear volume increased at 3, 7, 10d and after 10d, it was higher than that of blank group and PBS group, corneal epithelial cells were reduced	-
Lee[22], 2011	United States	EGCG	Flavanol	C57BL/6 female mice aged 7 to 8wk	Controlled environment chamber+subcutaneous atropine+scopolamine	Four groups: blank group, PBS group, 0.1%EGCG group, 0.01%EGCG group	3	Twice a day for 3-9d	Corneal fluorescein staining score	9d	Corneal staining was significantly reduced in all treated groups compared to the untreated group	No cytotoxic
Peng[23], 2010	China	Buddleja officinalis	Flavonoids	4wk Wistar males rats (about 200 g), ovariectomized	Castration to induce decreased androgen levels	Nine groups: A1-A3, sham-operated normal group, B1-B3, operated control group, C1-C3, Tremodendron extract drops treated group	5	Once a day for 3mo	Schirmer's test	1, 2, 3mo	There was a significant difference in Schirmer I test values in group C, which indicated that the basilar Schirmer secretion was significantly increased by the eye drops	-
Lee[24], 2023	Republic of Korea	PCE	Flavonoids	6wk Sprague-Daw ley male mice	Induction of dry stress *via* air exposure	Five groups: vehicle treatment group, 30,80, or 150 mg/kg PCE treatment group, omega-3 treatment group	5	Once a day for 7d	Phenol red thread test; corneal irregularity score; PAS staining; TUNEL assay	7d	Oral PCE restore tear secretion in a concentration-dependent manner, and the tear volume of the high concentration PCE group was restored to normal level, and the effect was stronger than omega-3; PCE reduce corneal surface damage and inflammatory response	-
Xiao[25], 2018	China	Daidzin	Isoflavone	8wk Wistar male rats (250-300 g)	Lacrimal gland excision	Four groups: vehicle control group, 0.1, 1, or 10 μmol/L daidzin group	-	Four times a day for 14d	-	14d	After removal of the lacrimal gland, tear secretion was reduced by approximately 45%, which was restored by daidzein	-
Xie[26], 2022	China	Acacetin	Isoflavone	C57BL/6 male mice	Applying two unpredictable stressors daily for 7 consecutive weeks, leading to depression-associated dry eye	Four groups: vehicle group, low dose of acacetin group, high dose of acacetin group, escitalopram group	-	Once a day for 21d	Phenol red thread test; fluorescein sodium staining; PAS staining	21d	Acacetin, but not escitalopram, reversed corneal defects and reduced tear secretion in mice. The the model mice were protected from dry eye disease by acacetin	-
Chiang[27], 2023	China	PSP	Flavonoids	4wk Sprague Dawley male rats	Fed a high-fat diet for 19wk to induce obesity and associated dry eye	Three groups: control group, PSP group, atorvastatin group	9	Once a day for 19wk	Schirmer's test; Lissamine green staining	19wk	PSP reverse the reduction of tear secretion, reduce the corneal epithelial damage caused by high-fat diet, and the effect is better than atorvastatin group	-
Kang[28], 2018	Republic of Korea	EERV	Flavonoids	6wk BALB/c male mice	Topical application of 0.2% benzalkonium chloride eye drops twice daily for 14 consecutive days	Four groups: control group, 10, 50 mg/kg EERV, positive control	6	Two times a day for 14d	Tear break-up time	14d	The positive control group and 50 mg/kg EERV group improved the irregularity of ocular surface and the changes of epithelial cells	-
Hong[29], 2020	Republic of Korea	AKE	Flavonoids	6wk BALB/c male mice	Intraperitoneal injection of scopolamine (2.5 mg/mL, twice daily)	Five groups: control group, vehicle control group, 10, 50, or 100 mg/kg AKE group	7	Once a day for 14d	Schirmer's test; corneal fluorescein staining score; H&E staining	14d	AKE increases tear secretion and tear film stability; AKE 50 and 100 mg/kg inhibited scopolamine-induced corneal damage, and AKE was beneficial to corneal epithelial cells	-
Inaba[30], 2022	Japan	Quercetin	Flavonol	6wk BKS mice	Used genetically diabetic model mice	Two groups: control group, quercetin group	10	5mo	Phenol red thread test	5mo	Lacrimal gland function of quercetin-treated mice was significantly higher than that of control mice	-
Nakamura[31], 2014	Japan	MBE	Flavonoids	8wk Sprague-Dawley female rats	Rats were placed on a suspended plastic tube swing, suppressing normal blinking	Three groups: MBE group, BCE group, BBE group	6	Once a day for 10d	Modified schirmer test; fluorescein sodium staining	11d	The tear secretion of MBE group was significantly higher than that of control group, and returned to near normal level at 11d; corneal fluorescein staining score in MBE group was lower than that in control group	-

EGCG: Epigallocatechin gallate; PCE: Purple corn extract; PSP: Purple sweet potato; EERV: Ethanol extract of *R. volubilis*; AKE: Aster koraiensis extract; MBE: Maqui berry extract; BCE: Blackcurrant berry extract; BBE: Bilberry extract; PBS: Phosphate buffer solution; DMSO: Dimethyl sulfoxide; PAS: Periodic acid-Schiff.

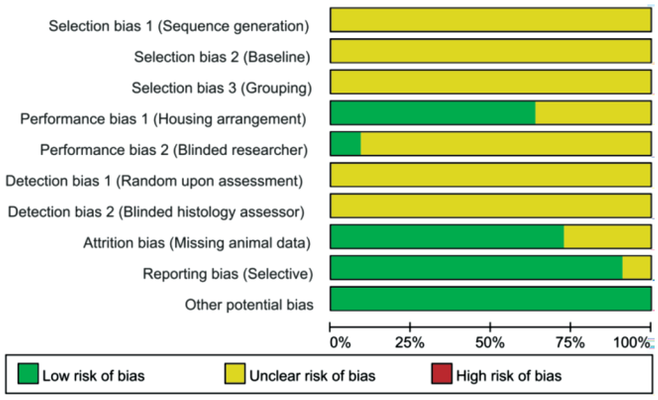

Figure 2 Risk of bias graph.

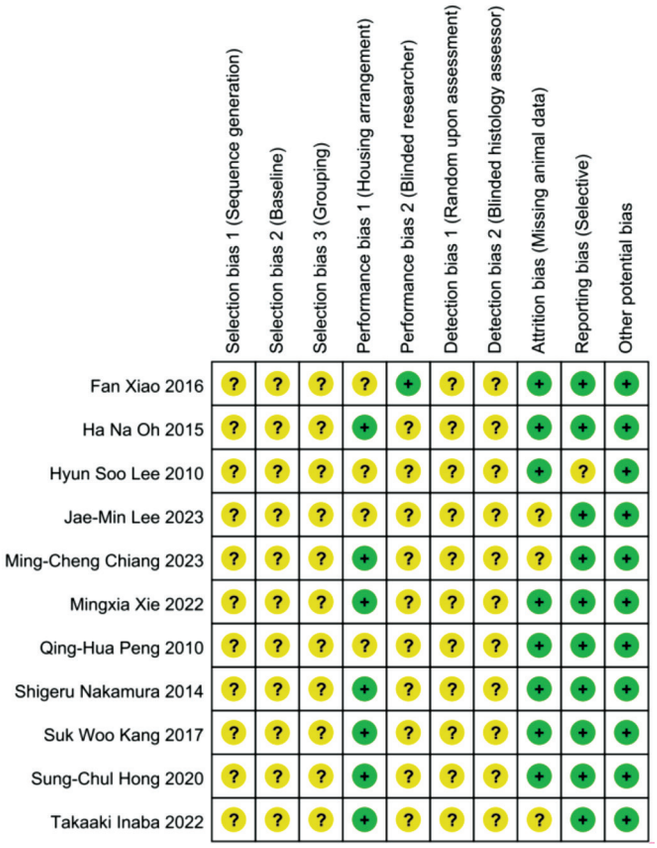

Figure 3 Risk of bias summary.

to the control group[28-29]. After a 10-day intervention with MBE[31], tear secretion in DED mice returned to nearly normal levels. Four studies[21,25-26,30] assessed the impact of a single flavonoid on tear secretion. Following treatment with quercetin eye drops (0.5%)[21,30], there was a significant increase in tear volume compared to the control group. Additionally, treatment with daidzin restored tear secretion in certain DED rat models[25]. After three weeks of intervention with a high dose of acacetin (15 mg/kg), tear secretion in mice was found to be significantly increased[26].

Effects of Flavonoid Rich Substances and Flavonoids on Corneal Epithelial Damage Four studies[27-29,31] have examined the enhancement of corneal epithelial repair through the application of flavonoid-rich substances. PSP has been shown to significantly reduce corneal epithelial damage in rat models[27].

Following oral administration of the EERV at a dosage of 50 mg/kg for a duration of two weeks, there was a notable inhibition of corneal inflammation in a dry eye disease mouse model[28]. Additionally, this treatment helped maintain corneal epithelial integrity by reducing squamous metaplasia. Intervention with AKE at doses of 50 and 100 mg/kg over a duration of 2wk significantly mitigated scopolamine-induced damage to the corneal epithelium[29]. After a 10-day intervention with MBE[31], the corneal fluorescein staining score was significantly lower in comparison to the control group, indicating that maqui berry extract effectively reduced corneal surface damage. Four studies[21-22,25-26] examined the impact of flavonoids on corneal epithelial damage. Quercetin has been shown to decrease the expression of pro-inflammatory factors in the lacrimal gland, mitigate the shedding of corneal epithelial cells, and consequently reduce corneal epithelial damage[21]. EGCG has been shown to play a significant role in reducing the infiltration of corneal $CD11b^{+}$ inflammatory cells by down-regulating the expression of IL-1β and C-C motif chemokine ligand 2 (CCL2) mRNA[22]. Acacetin demonstrates potential in improving corneal epithelial injury in murine models[26]. Daidzin exerts protective effects against corneal oxidative stress and reduces cell apoptosis through the scavenging of tyrosyl free radicals[25].

DISCUSSION

The objective of this study was to investigate the therapeutic effects of flavonoids on DED. Currently, the management of DED-related symptoms primarily relies on palliative measures, highlighting an urgent need for intervention strategies grounded in pathophysiology. This systematic review synthesized evidence from 11 animal studies and demonstrated that flavonoids, a class of polyphenolic compounds abundantly found in plants, can alleviate dry eye symptoms through various mechanisms including immune inflammation, oxidative stress modulation, hormone signaling pathways, and apoptosis[32-33].

Modulation of Immune and Inflammatory Responses A key finding of this review is the significant anti-inflammatory activity of flavonoids, which target both innate and adaptive immunity in DED[34]. At the molecular level, flavonoids have been shown to inhibit key pro-inflammatory signaling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and c-Jun N-terminal kinase (JNK)/p38 mitogen-activated protein kinase (MAPK). This inhibition leads to a reduction in the expression levels of cytokines and chemokines that contribute to glandular inflammation[35]. For instance, quercetin has been shown to effectively inhibit the nuclear translocation of the NF-κB p65

subunit in lacrimal gland tissue. This action contributes to the alleviation of corneal epithelial inflammation and a reduction in leukocyte infiltration[21]. Similarly, PCE significantly reduces mRNA expression levels of pro-inflammatory cytokines including IL-1β, IL-6, TNF-α, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS)[24]. AKE modulates the NF-κB signaling pathway by downregulating TNF-α, IL-6, and IL-1β while simultaneously enhancing antioxidant enzyme expression through the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway. This dual action mitigates oxidative stress in corneal epithelial cells and improves tear film stability[29]. In the context of adaptive immune regulation, flavonoids play a significant role in modulating the balance between Th17 and Treg cells. For instance, genistein, a well-known isoflavone, has been shown to upregulate *Foxp3* gene expression and facilitate the differentiation of Treg cells. Additionally, it inhibits IL-17 secretion from Th17 cells and reduces lymphocyte infiltration in salivary glands[36]. The *Buddleja officinalis* of action of the extract is involved in down-regulating the expression of B-cell activating factor (BAFF) receptor, effectively inhibiting the proliferation of abnormal B cells and the production of autoantibodies[23].

Alleviation of Oxidative Stress Flavonoids exert a protective effect by activating the Nrf2/HO-1 antioxidant pathway, thereby enhancing the activities of superoxide dismutase, catalase, and glutathione peroxidase to eliminate reactive oxygen species[37]. Daidzin modulates the oxidative stress response by influencing the expression of HO-1. Furthermore, daidzein directly inhibits the activity of prostaglandin synthetase, and its significant free radical scavenging capacity has been validated through electron spin resonance assays. EGCG potently inhibits the expression of IL-1β and monocyte chemotactic protein-1[22]. PSP exerted its effects through multiple pathways. It alleviated oxidative stress by increasing the level of superoxide dismutase-1 and reducing hypoxia-inducible factor-1[27]. MBE have been shown to enhance the activity of mitochondrial complex IV, sustain ATP production in lacrimal gland cells, and provide protection against apoptosis induced by oxidative stress[31]. Quercetin reduces cytochrome c release and caspase-9 activation by inhibiting the excessive production of reactive oxygen species (ROS) mediated by complex I[38].

Regulation of Hormone Signaling Hormone imbalance aggravates the symptoms of DED, and flavonoids can act as selective receptor modulators to restore balance. The extract of *Buddleja officinalis* can upregulate the expression of androgen receptor in lacrimal gland of castrated rats, and restore androgen-dependent tear secretion by enhancing the membrane localization of aquaporin 3 and chloride channels[23]. Furthermore, beyond modulating androgenic pathways, flavonoids also regulate estrogenic signaling. Genistein, a major isoflavone, preferentially binds estrogen receptor α in salivary glands—which share functional homology with lacrimal glands. This binding inhibits estrogen receptor α (ER-α) recruitment of coactivator steroid receptor coactivator 3 (SRC-3), downregulating Acyl-CoA synthetase long-chain family member 4 (ACSL4), a key ferroptosis regulator[39].

Inhibition of Cell Death and Promotion of Cellular Homeostasis Flavonoids have the capacity to regulate various cell death pathways, including apoptosis, pyroptosis, and ferroptosis, thereby playing a crucial role in maintaining the integrity of glandular epithelium. For instance, the acacetin exerts its protective effects primarily by targeting the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome. acacetin promotes gp78/Insig-1-mediated ubiquitination and degradation of NLRP3, which effectively suppresses inflammasome activation and the subsequent pyroptotic cell death in corneal epithelial cells. This upstream inhibition leads to reduced levels of key inflammatory cytokines such as IL-1β and IL-18, thereby alleviating ocular surface damage[26]. The compound is also capable of upregulating the autophagy-related protein microtubule-associated proteins 1A/1B light chain 3 (LC3) II/LC3I ratio, thereby promoting the removal of dysfunctional mitochondria and maintaining cellular energy homeostasis[35].

Although the majority of evidence presented in this review is derived from mouse and rat models, therapeutic benefits of flavonoids have also been observed in rabbit dry eye models, thereby supporting their translational potential across species. For instance, Tseng *et al*[40] reported that artificial tears containing EGCG and hyaluronic acid significantly improved tear secretion, reduced corneal epithelial damage, and decreased levels of inflammatory mediators such as IL-6, IL-8, and TNF-α in corneal tissues. These effects are likely attributed to the anti-inflammatory and mucoadhesive properties of these compounds. In a separate study, Luo and Lai[41] developed a novel thermosensitive *in situ* gel loaded with EGCG that enhanced ocular surface integrity, reduced tear evaporation, suppressed corneal epithelial apoptosis, and preserved conjunctival goblet cell function along with mucin 5AC (MUC5AC) expression. Characterized by their larger eye size, rabbit models facilitate repeated clinical evaluations and strengthen the evidence for the efficacy of flavonoids in treating DED. Furthermore, the consistency between data from rabbit and rat studies underscores the multi-mechanistic actions of flavonoids, which encompass anti-inflammatory, antioxidant, and anti-apoptotic pathways.

The strengths of this study lie in the fact that all included studies were randomized controlled trials, demonstrating a

high overall quality. Furthermore, these studies originated from four different countries, which enhances the reliability of the results. However, this study is not without its limitations. The search strategy employed was restricted to articles published in Chinese and English, which may have resulted in the omission of significant studies and could potentially influence the overall analysis of the results. In addition, a quantitative Meta-analysis could not be conducted due to significant heterogeneity among the included studies. This heterogeneity is primarily evident in two aspects: the timing of outcome measurement and the intervention protocols, which encompass various types of flavonoids and different routes of administration. These methodological differences hindered statistical pooling of data, preventing the derivation of uniform effect sizes. Consequently, our analysis remains narrative in nature, with conclusions drawn from qualitative synthesis.

CONCLUSION

Through the integration of 11 animal studies, this systematic review demonstrates that flavonoids can significantly alleviate symptoms of dry eye, enhance tear secretion, and reduce corneal epithelial damage. This systematic review highlights several critical avenues for future research. Subsequent studies should focus on clarifying the structure-activity relationships across different flavonoid subclasses to pinpoint the most therapeutically promising compounds. It will also be essential to use specific pathway inhibitors to functionally verify the mechanisms identified herein and establish causal relationships. Additionally, composite flavonoid formulations deserve further exploration, as they may better mimic the natural synergistic effects observed in plant extracts; investigating their interactive mechanisms and potential synergies represents a valuable research direction. From a translational perspective, our results support the development of flavonoids as active ingredients in ophthalmic formulations. Future work should emphasize pharmacokinetic and safety studies in larger animal models and humans to determine optimal dosing protocols. Ultimately, rigorously designed clinical trials will be crucial to confirm the efficacy and safety of selected flavonoids in treating dry eye disease, facilitating their integration into mainstream therapeutic strategies.

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Authors' Contributions: Lyu J proposed the subject of this study. A comprehensive literature search and data extraction were conducted by Lyu J and Jiao SY to assess the risk of bias in included studies. The data analysis was performed by Lyu J and Zhang Q, while the paper writing was solely undertaken by Lyu J. Subsequently, the paper underwent a thorough review and revision process involving Zhang Q, Jiao SY, Li WJ, and Ding S before being approved by all authors.

Data Availability Statement: The original data involved in the manuscript can be obtained from the references.

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Conflicts of Interest: Lyu J, None; **Jiao SY,** None; **Li WJ,** None; **Ding S,** None; **Zhang Q,** None.

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