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PRECLINICAL EFFICACY OF DIOSMIN AND HESPERIDIN IN CHRONIC VENOUS INSUFFICIENCY: A COMPARATIVE REVIEW

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The purpose of the study was to systematize data on the preclinical efficacy of diosmin and hesperidin in chronic venous insufficiency and to evaluate these substances in a comparative context. This review highlights that while diosmin and hesperidin share several beneficial properties in the treatment of chronic venous insufficiency, their distinct pharmacological profiles contribute to complementary therapeutic effects. Diosmin is mainly responsible for improving venous contractility and reducing inflammatory remodeling, whereas hesperidin primarily protects the endothelium and alleviates oxidative stress. Their combined use leads to a more comprehensive treatment strategy and underscores the potential for next-generation phlebotonic therapies that leverage the synergistic benefits of these natural compounds.

Key words: venotonics, diosmin, hesperidin, preclinical studies, chronic venous insufficiency, phleboprotective effect.

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ДОКЛІНІЧНА ЕФЕКТИВНІСТЬ ДІОСМІНУ ТА ГЕСПЕРИДИНУ ПРИ ХРОНІЧНІЙ ВЕНОЗНІЙ НЕДОСТАТНОСТІ: ПОРІВНЯЛЬНИЙ ОГЛЯД

Метою дослідження було систематизувати дані щодо доклінічної ефективності діосміну та гесперидину при хронічній венозній недостатності та оцінити ці речовини в порівняльному контексті. Цей огляд підкреслює, що хоча діосмін і гесперидин мають низку спільних корисних властивостей у лікуванні хронічної венозної недостатності, їхні відмінні фармакологічні профілі сприяють взаємодоповнюючим терапевтичним ефектам. Діосмін переважно відповідає за покращення венозної скоротливості та зменшення запального ремоделювання, тоді як гесперидин головним чином захищає ендотелій і зменшує оксидативний стрес. Їхнє комбіноване використання забезпечує більш комплексну стратегію лікування та підкреслює потенціал флеботонічних терапій наступного покоління, які використовують синергетичні переваги цих природних сполук.

Ключові слова: венотоніки, діосмін, гесперидин, доклінічні дослідження, хронічна венозна недостатність, флебопротекторний вплив.

The study is a fragment of the research work "Development and implementation of innovative technologies in the treatment and prevention of violations of the integrity and patency of blood vessels in wartime conditions", state registration No. 0123U100204.

Chronic venous insufficiency (CVI) is a multifactorial vascular disorder characterized by impaired venous return, increased venous pressure, capillary leakage, and ultimately, clinical manifestations such as leg edema, pain, and skin changes [1, 2, 4, 15, 32, 34]. Over the past decades, naturally derived flavonoids have been investigated extensively for their capacity to counteract the pathological processes underlying CVI [6, 7, 18, 31]. Two of the most studied flavonoids in this context are diosmin and hesperidin, both of which originate from citrus fruits [9, 27]. Preclinical investigations have focused on elucidating the mechanisms by which these compounds improve venous tone, suppress inflammatory cascades, and enhance microcirculatory function. In particular, diosmin's ability to improve venous contractility and

reduce endothelial permeability has been a consistent finding in various animal models, while hesperidin appears to exert predominantly antioxidant and endothelial-protective actions. Together, these agents not only offer complementary modes of action but also form the basis for combination therapies such as micronized purified flavonoid fraction (MPFF), which has shown promising results in the treatment of CVI [5, 22].

The purpose of the study was to systematize data on the preclinical efficacy of diosmin and hesperidin in chronic venous insufficiency and to evaluate these substances in a comparative context.

To conduct a comprehensive scientific review a systematic literature search was performed across multiple scientometric databases, including PubMed for biomedical and life sciences literature, Scopus for multidisciplinary scientific articles and conference proceedings, Web of Science for high-impact journals and citation analysis, and Google Scholar to capture additional grey literature and open-access studies. The search aimed to identify relevant preclinical studies, including in vitro, in vivo, and animal model research, evaluating the efficacy of diosmin and hesperidin in the context of chronic venous insufficiency. A combination of controlled vocabulary, such as MeSH terms in PubMed, and free-text keywords like “Diosmin”, “Hesperidin”, “Chronic Venous Insufficiency”, “Preclinical”, “Efficacy”, “Animal model”, “In vitro”, and “In vivo” was used to maximize the retrieval of relevant studies. Boolean operators were applied, using AND to combine terms related to diosmin/hesperidin and chronic venous insufficiency, OR to include synonyms or related terms like “venous insufficiency” or “venous disease”, and NOT to exclude clinical studies or unrelated conditions, such as “clinical trial” or “human study”. The search process involved an initial query of each database using predefined search terms, with filters for publication date from 2020 to 2025 to ensure relevance and for English language. The last search was conducted on July 20, 2025.

The verbatim search queries used in each database were as follows:

– PubMed: (“Diosmin”[Mesh] OR “Hesperidin”[Mesh] OR “Diosmin” OR “Hesperidin”) AND (“Venous Insufficiency”[Mesh] OR “chronic venous insufficiency” OR “venous insufficiency” OR “venous disease”) AND (“preclinical” OR “animal model” OR “in vitro” OR “in vivo” OR “efficacy”) NOT (“clinical trial” OR “human study” OR “clinical” OR “human”) (Filters applied: Publication date from 2020/01/01 to 2025/07/20; Language: English).

– Scopus: TITLE-ABS-KEY ((diosmin OR hesperidin) AND (“chronic venous insufficiency” OR “venous insufficiency” OR “venous disease”) AND (preclinical OR “animal model” OR “in vitro” OR “in vivo” OR efficacy)) AND NOT (“clinical trial” OR “human study” OR clinical OR human) AND PUBYEAR > 2019 AND PUBYEAR < 2026 AND (LIMIT-TO (LANGUAGE , “English”).

– Web of Science: TS=(diosmin OR hesperidin) AND TS=(“chronic venous insufficiency” OR “venous insufficiency” OR “venous disease”) AND TS=(preclinical OR “animal model” OR “in vitro” OR “in vivo” OR efficacy) NOT TS=(“clinical trial” OR “human study” OR clinical OR human) (Filters applied: Timespan: 2020-2025; Languages: English).

– Google Scholar: “diosmin” OR “hesperidin” “chronic venous insufficiency” OR “venous insufficiency” OR “venous disease” “preclinical” OR “animal model” OR “in vitro” OR “in vivo” OR “efficacy” -“clinical trial” -“human study” -clinical -human (Filters applied: Custom range: 2020–2025; Language: English).

Studies were included if they were published in peer-reviewed journals, focused on preclinical research, investigated the efficacy of diosmin, hesperidin, or their combination in CVI, and were published in English or had an English translation available. Studies were excluded if they were clinical trials, human studies, focused on unrelated conditions or compounds, or were reviews, editorials, or non-original research articles.

Potentially relevant articles were retrieved for full-text evaluation to confirm eligibility and extract data on study design, outcomes, and findings (Table 1).

Table 1

Simplified PRISMA Flow

Stage	Description	Number of Records/Studies
1. Identified	Total number of records identified through database searching and other sources	325
2. Duplicates Removed	Number of records removed before screening (e.g., duplicates)	156
3. Screened (Title/Abstract)	Number of records screened after duplicates were removed	169
4. Assessed for Eligibility (Full-text)	Number of full-text articles assessed for eligibility against the inclusion/exclusion criteria	100
5. Included in Review	Total number of primary studies finally included in the systematic review	36

As shown in Table 1, out of 325 identified literature sources, we selected 169, of which 100 were full-text and met the selection criteria. Table 2 provides a description of the studies from the literature list that were the author's preclinical studies, based on which we systematized the data in the results.

Table 2

Summary table of study characteristics

Author, Year	Study Type (in vitro, in vivo)	Model / Animal Species	Dose and Duration of Treatment	Main Outcome Observed
Casili G, 2021	In vitro, ex-vivo, in vivo	HUVECs (in vitro); mesenteric vessels (ex-vivo); saphene vein ligation model of CVI (in vivo, animal species unspecified)	Not specified (flavonoid compound containing diosmin)	Reduced varicose vein pathophysiology; regularized venous tone; improved absorption, contractile activity, and reduced inflammation (via VEGF, CD34, and inflammatory mediators)
Cyrino FZ, 2021	In vivo	Hamsters (external right iliac vein ligation model)	MPFF (containing diosmin) 100 mg/kg/day, 7 days	Prevented leukocyte adhesion to microvalves; reduced venular diameter; inhibited microvalve inflammation
Guo Z, 2022	In vivo	Mice (iliac vein stenosis model)	Diosmin 20-40 mg/kg (duration unspecified)	Alleviated venous permeability and endothelial dysfunction (e.g., maintained ZO-1); suppressed inflammation (reduced IL-1α, IL-6, MCP1); improved muscle function via actin organization and contraction
Gwozdzinski L, 2023	In vitro	Human endothelial cells (HUVEC and HVVEC from varicose veins)	Diosmin 1-10 mg/mL (duration unspecified)	Reduced membrane fluidity and adhesive properties of HVVEC; lowered risk of thrombosis; slight superoxide release
Kusumo MHB, 2024	In vivo	Mice (croton oil-induced hemorrhoids model)	MPFF (diosmin-hesperidin, dose unspecified)	Reduced tissue edema and inflammation; decreased TNF-α and MMP-9; increased TGF-β and collagen I/III ratio; accelerated wound healing
Pinna C, 2021	In vitro	Human umbilical vein (HUV)	Hesperidin and diosmin (concentrations unspecified)	Hesperidin more potent than diosmin as vasorelaxant; improved vascular and endothelial function; KATP channel activation in hesperidin vasorelaxation
Wójciak M, 2022	In vitro	Endothelial cells	Diosmin/diosmetin (concentrations unspecified)	Ameliorated H2O2-induced oxidative stress; restored antioxidant enzymes (SOD, CAT, GPx); lowered MDA levels
Zou J, 2022	In vivo	Mice (iliac vein stenosis model of venous obstruction)	Diosmin 40 mg/kg (duration unspecified)	Alleviated vascular leakage and inflammation; reduced ICAM-1, VCAM-1, IL-1α, IL-6, MCP-1

Mechanisms of Action.

The efficacy of diosmin in preclinical models of CVI is primarily attributed to its venotonic, anti-inflammatory, and antioxidant properties. Diosmin increases venous wall contractility by enhancing the calcium sensitivity of vascular smooth muscle cells, thereby promoting improved venous tone and reducing venous stasis [22]. In addition, diosmin decreases capillary permeability and facilitates lymphatic drainage, leading to a reduction in edema. At a molecular level, several studies have shown that diosmin decreases the release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), modulates the expression of adhesion molecules like ICAM-1 and VCAM-1, and inhibits the expression of matrix metalloproteinases (MMP-2 and MMP-9), all of which contribute to reduced inflammation and favorable remodeling of the venous wall [3, 11, 16, 35, 36,]. Diosmin also exerts antioxidant effects by upregulating endothelial nitric oxide synthase (eNOS) expression and stimulating antioxidant defenses, thereby mitigating oxidative stress within the venous microenvironment [3, 21, 25, 30, 33].

In contrast, hesperidin, which is often administered as a minor component (typically 10 %) within combination formulations appears to provide mainly endothelial protection and antioxidant activity. Preclinical studies have demonstrated that hesperidin reduces reactive oxygen species (ROS) production and lipid peroxidation, leading to the preservation of endothelial integrity [8, 14, 21, 22, 25, 33]. In inflammatory settings, hesperidin decreases the levels of pro-inflammatory mediators and downregulates adhesion molecules, resulting in attenuated leukocyte adhesion and reduced microvascular inflammation [14, 24]. Although both flavonoids share overlapping properties, the principal difference lies in their target profiles: diosmin acts predominantly on smooth muscle and overall venous wall integrity, whereas hesperidin largely ensures the preservation of endothelial function and capillary stability [14, 25, 35].

Preclinical Studies on Diosmin.

A substantial body of preclinical research has demonstrated that diosmin is efficacious in models of CVI. In animal studies, diosmin has been shown to lower limb circumference and reduce venous valve

dilatation, with one experimental model in rats showing that oral administration over 21 days not only prevented disease progression but also preserved venous blood flow velocity [3]. Other investigations using isolated rat venous segments have revealed that diosmin enhances sympathetic-mediated contraction and increases the sensitivity of the vascular smooth muscle contractile apparatus to calcium ions, which directly contributes to improved venous tone [22]. In addition to these hemodynamic improvements, diosmin consistently reduces endothelial permeability and leukocyte extravasation in animal models, thereby dampening the inflammatory cascade that is central to CVI pathology [3, 5, 11, 36].

Diosmin's beneficial effects are not limited to functional hemodynamic improvements. Histopathological assessments in preclinical models have found that diosmin treatment leads to a reduction in inflammatory cellular infiltration and decreased extracellular matrix remodeling, as evidenced by lower MMP expression and diminished collagen deposition. Such effects not only prevent the deterioration of venous valves but also contribute to the stabilization of the venous wall over time [35]. Moreover, preclinical toxicology studies have verified that diosmin is safe and well-tolerated in animal models, with doses up to 300 mg/kg/day administered over extended durations showing no significant adverse effects, thereby underscoring its favorable safety profile [29].

In addition, sophisticated preclinical investigations have demonstrated that diosmin administration leads to a downregulation of pro-inflammatory transcription factors such as NF-κB and upregulation of protective molecules like eNOS and PPAR-γ [19]. These molecular events collectively contribute to the restoration of vascular homeostasis by mitigating oxidative stress and modulating the inflammatory response [5, 30]. Such findings confirm that diosmin's multifaceted mechanism of action makes it a potent agent for improving venous function in chronic venous insufficiency [20, 35].

Preclinical Studies on Hesperidin.

Preclinical studies on hesperidin have focused on its antioxidant, anti-inflammatory, and endothelial-protective roles. Although hesperidin is typically evaluated in combination with diosmin as part of MPFF, preclinical models have also examined its individual effects. Hesperidin has been shown to reduce oxidative stress by decreasing ROS production and limiting lipid peroxidation in vascular tissues. In rodent models subjected to ischemia-reperfusion injury, treatment with hesperidin resulted in improvements in capillary resistance and a reduction in inflammatory mediators, both of which are critical for maintaining microcirculatory integrity [14, 22].

Furthermore, hesperidin has been documented to decrease levels of nitric oxide and inflammatory cytokines such as IL-6, thereby attenuating endothelial dysfunction – a hallmark of CVI. By reducing the expression of adhesion molecules and limiting leukocyte-endothelial interactions, hesperidin plays an essential role in preventing microvascular leakage and the subsequent tissue edema that is characteristic of chronic venous disorders [14, 24]. Many in vitro studies have confirmed these findings, demonstrating that hesperidin preserves endothelial cell viability under oxidative stress conditions and modulates gene expression profiles associated with inflammation and vascular repair [14, 22].

Preclinical investigations also highlight hesperidin's beneficial impact on microvascular parameters. Studies have reported improvements in capillary blood flow and a reduction in capillary fragility when hesperidin-containing formulations are administered, suggesting that hesperidin not only protects the endothelium but also enhances overall microcirculatory function [24]. These effects are particularly important in CVI, where compromised endothelial function contributes significantly to disease pathology [14]. Although the individual contribution of hesperidin may be less pronounced than that of diosmin regarding venotonic effects, its ability to stabilize the endothelial barrier and reduce oxidative damage is a critical component of the overall therapeutic profile observed in combined formulations [22, 28].

Pharmacokinetic and Formulation Advances.

One of the challenges associated with the therapeutic use of diosmin is its inherent poor solubility and bioavailability. Advances in formulation technology, such as micronization and the use of phytosomal or nanoparticle delivery systems, have significantly enhanced the absorption of diosmin, leading to improved systemic exposure and therapeutic efficacy in preclinical models [30, 35]. Although hesperidin is less affected by these limitations, its incorporation into combination products alongside improved diosmin formulations optimizes the overall pharmacokinetic profile of the therapy. These advancements not only ensure that the active metabolites reach their target sites in sufficient concentrations but also contribute to a more consistent clinical response [28, 35].

Safety and Tolerability in Preclinical Models.

An essential aspect of preclinical evaluation concerns the safety and tolerability of candidate therapeutics. Diosmin has been extensively studied in toxicology models, demonstrating an excellent safety profile with no significant adverse effects even at high doses administered over several weeks [29].

Hesperidin similarly exhibits a favorable safety profile, with animal studies reporting minimal toxicity and no significant side effects. These findings are critical given the chronic nature of venous insufficiency, which necessitates long-term treatment regimens [14, 26]. The combined use of these agents in MPFF formulations has not resulted in any new safety concerns, further supporting their utility as safe and effective treatments for CVI.

Metabolic Pathways and Formulation Considerations.

Both diosmin and hesperidin undergo extensive metabolism following oral administration, which plays a crucial role in their efficacy. Diosmin is converted into its active aglycone, diosmetin, which is further conjugated into glucuronide derivatives such as diosmetin-3-O-glucuronide – the major circulating metabolite responsible for many of its biological effects [35]. In particular, diosmetin-3-O-glucuronide is recognized as a key metabolite mediating the venotonic and anti-inflammatory effects observed in preclinical studies. In contrast, hesperidin is metabolized into the aglycone hesperetin and subsequently conjugated, with these metabolites mediating its vascular protective actions. Advanced formulation technologies, including micronization and the use of nanocarriers, have been shown to significantly improve the bioavailability of diosmin, thereby enhancing its therapeutic efficacy in the treatment of CVI [30, 35]. Although hesperidin does not typically face the same bioavailability challenges, its incorporation into standardized extracts and combination formulations further optimizes its delivery and effectiveness [28].

Molecular Targets, Gene Expression, and Adhesion Molecules.

Recent preclinical studies have provided insight into the molecular targets of diosmin and hesperidin. Diosmin has been shown to modulate the expression of transcription factors, such as peroxisome proliferator-activated receptor gamma (PPAR-γ), which plays an important role in regulating angiogenesis and inflammatory responses in venous tissues [5]. This modulation contributes to a reduction in extracellular matrix remodeling and a stabilization of venous valve structure. Additionally, diosmin's inhibitory effects on NF-κB activation reduce the overall inflammatory burden within the venous system [3]. Hesperidin, while also influencing inflammatory pathways, has been particularly noted for its capacity to upregulate genes involved in the antioxidant defense system – such as nuclear factor erythroid 2–related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) – thereby protecting endothelial cells from oxidative damage [14, 26]. The modulation of these molecular targets underlies the observed reduction in inflammation and improvement in vascular function, and the dual targeting of both adhesion molecules and gene expression pathways by these flavonoids is indicative of their comprehensive therapeutic potential [14, 26]. The dual modulation of adhesion molecules and gene expression by these agents is central to their ability to restore vascular homeostasis and may explain the superior outcomes observed in combination therapy [22, 24].

In Vivo Versus In Vitro Correlations.

The preclinical studies encompass both in vivo animal models and in vitro experiments using isolated human venous tissues and cultured endothelial cells. In vivo models – ranging from rat models of saphenous vein hypertension to hamster cheek pouch preparations – have consistently demonstrated that diosmin improves venous hemodynamics and reduces inflammatory markers [3]. In vitro studies have reinforced these findings by showing that diosmin enhances norepinephrine-induced venous contractions and that hesperidin protects endothelial cells against oxidative insult, preserving their function and integrity [14, 22]. The strong correlation between the in vivo and in vitro data supports the translational relevance of these findings, suggesting that the mechanisms observed in controlled experimental conditions are likely to be replicated in clinical settings [22, 24].

Comparative Analysis of Diosmin and Hesperidin.

A direct comparison of the preclinical evidence reveals both shared and distinct features in the modes of action of diosmin and hesperidin. Diosmin demonstrates robust venotonic effects that are essential for improving the mechanical function of the venous wall. Its capacity to enhance calcium sensitivity in vascular smooth muscle cells directly translates into increased venous contractility and reduced venous distensibility, which helps to prevent venous stasis and lower limb edema [22]. In parallel, diosmin effectively reduces capillary leakage and leukocyte adhesion by modulating pro-inflammatory cytokines and adhesion molecules, further contributing to its anti-inflammatory and anti-edematous actions [3, 35].

Hesperidin, on the other hand, appears to exert its effects predominantly at the level of the endothelium. It shows significant antioxidant activity by limiting ROS production and reducing nitric oxide levels, which protects the microvasculature from oxidative injury. Hesperidin's anti-inflammatory properties are manifested through the downregulation of cytokines such as IL-6 and suppression of adhesion molecule expression, which in turn preserves capillary integrity and enhances microvascular blood flow [14, 22]. Although hesperidin alone may not exhibit as strong a venotonic action as diosmin, the endothelial protection it provides is indispensable in counteracting the biochemical and cellular mechanisms that underlie chronic venous insufficiency [24].

One of the most compelling aspects of the preclinical data is the demonstration of synergy between diosmin and hesperidin when used in combination. The widely used MPFF formulations, which contain approximately 90 % diosmin and 10 % hesperidin, have been shown to yield superior outcomes compared to single-agent therapy. In animal models, combination treatment has resulted in a greater reduction in limb circumference, improved venous emptying times, and enhanced capillary resistance when compared to diosmin or hesperidin alone [22, 28]. The synergy likely arises because diosmin's strong venotonic and anti-inflammatory actions are complemented by hesperidin's endothelial-stabilizing and antioxidant effects; together, they target both the mechanical and biochemical dysfunctions present in CVI [22]. Moreover, formulations that enhance the bioavailability of diosmin, such as those employing micronization techniques, ensure that the venotonic benefits of diosmin are fully realized, even when used in combination with hesperidin [30, 35].

Safety profiles for both compounds have been favorable in preclinical assessments. Diosmin has been thoroughly tested in animal toxicology studies with high doses showing no significant adverse effects, while hesperidin has similarly demonstrated an excellent safety record with minimal toxicological concerns [14, 29]. The low incidence of side effects, coupled with the potent pharmacological actions observed, supports the therapeutic potential of these agents in chronic venous insufficiency [26, 35].

Combination Therapy and Synergistic Effects.

A critical observation from preclinical research is that the combination of diosmin and hesperidin yields synergistic benefits that exceed the effects of either agent alone. When administered together in MPFF formulations, diosmin's potent effects on venous wall contractility and anti-inflammatory activity are complemented by hesperidin's ability to enhance endothelial stability and reduce oxidative stress. This synergy is reflected in multiple endpoints, including reduced limb circumference, improved venous emptying times, and enhanced capillary integrity [8, 22, 28]. The cooperative interactions between the two compounds suggest that combination therapy addresses both the structural deficits and the biochemical imbalances inherent in chronic venous insufficiency – a dual action that is likely responsible for the superior clinical performance observed in these formulations [22, 24].

Combination Therapy and Clinical Implications.

The integration of diosmin and hesperidin into combination therapies reflects a rational strategy to address the multifactorial nature of chronic venous insufficiency. Preclinical studies have consistently demonstrated that MPFF formulations, which combine approximately 90 % diosmin with 10 % hesperidin, lead to a more comprehensive improvement in venous function. Such combination therapy not only enhances venous tone and reduces edema but also provides robust protection against endothelial dysfunction and oxidative damage [17, 21, 22, 28]. These synergistic effects are believed to arise from the complementary mechanisms of action where diosmin predominantly improves smooth muscle contractility and hesperidin safeguards the microvasculature through its antioxidant properties. The encouraging preclinical data have paved the way for subsequent clinical investigations that have corroborated the benefits observed in animal models [5, 10, 13, 14, 23].

Personalized Medicine and Future Perspectives.

Emerging trends in personalized medicine suggest that treatment strategies for chronic venous insufficiency might be further optimized by tailoring therapy to the individual patient's vascular profile. Given the distinct yet complementary mechanisms of action of diosmin and hesperidin, patients with predominant venous dilatation and smooth muscle dysfunction could particularly benefit from the venotonic effects of diosmin. Conversely, those exhibiting marked endothelial dysfunction and oxidative stress might derive greater benefit from hesperidin's protective actions. Future preclinical studies should aim to stratify models of CVI based on these parameters and evaluate the efficacy of tailored combination therapies. Such an approach could lead to more effective individualized treatment protocols and improved clinical outcomes [14, 29].

Limitations and Future Research Directions.

Despite the extensive preclinical evidence supporting the efficacy of diosmin and hesperidin, some limitations exist. Variability in experimental models, dosing regimens, and treatment durations among different studies can complicate direct comparisons and extrapolation to the human condition [22, 35]. Furthermore, while combination therapy appears advantageous, the precise molecular mechanisms underlying the synergistic interactions between diosmin and hesperidin remain incompletely understood and require further detailed investigation. Future research should aim to standardize preclinical protocols, employ more sophisticated animal models that closely mimic human CVI, and use comprehensive molecular analyses to delineate the specific signaling pathways involved. Advancements in formulation science to further enhance bioavailability, particularly for diosmin, and studies that explore the long-term

safety of these compounds in chronic administration settings will be essential to bridge the gap between preclinical efficacy and clinical application [30, 35].

Conclusion

In summary, the preclinical evidence strongly supports the efficacy of both diosmin and hesperidin in targeting the multifaceted pathophysiology of chronic venous insufficiency. Diosmin demonstrates pronounced venotonic and anti-inflammatory actions by enhancing smooth muscle contractility, reducing capillary leakage, and modulating critical inflammatory pathways. Hesperidin, by contrast, primarily exerts antioxidant effects and protects the endothelium from oxidative damage, thereby preserving microvascular function. When used in combination – as in MPFF formulations – the complementary actions of these flavonoids produce synergistic benefits that address both structural and functional abnormalities in the venous system. Advances in formulation technology have further improved the bioavailability of diosmin, ensuring that its potent pharmacological effects are fully realized. The favorable safety profiles of both agents, as demonstrated in various preclinical toxicology studies, underscore their potential for long-term use in patients with CVI. Although variability in experimental designs and the need for further elucidation of molecular mechanisms remain challenges, the comprehensive preclinical data provide a robust foundation for the continued development of diosmin and hesperidin as effective therapeutic agents for chronic venous insufficiency. Overall, the integration of these compounds into combination therapy represents a promising strategy for improving venous function, mitigating inflammation, and ultimately enhancing the quality of life for patients suffering from this chronic condition.

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