

RESEARCH ARTICLE

Nanoparticle-formulated quercetin-like compounds from *Dendrophthoe pentandra* promote vascular recovery in a rabbit model of experimentally modified ischemic-limb compression



Mochamad Lazuardi¹ , Hani Plumeriastuti² , Eka Pramytha Hestianah³ , Chi-Hsien Chien⁴ , Jie-Long He⁴ , and Qonita Kurnia Anjani⁵ 

1. Sub-division of Veterinary Pharmacy Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.
2. Sub-division of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.
3. Sub-division of Veterinary Histology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.
4. Department of Post-Baccalaureate Veterinary Medicine, Asia University, Taichung, Taiwan.
5. School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom.

ABSTRACT

Background and Aim: Quercetin-like compounds (QLCs) derived from *Dendrophthoe pentandra* growing on *Lansium domesticum* have potential endothelial-protective and vascular restorative properties, but their therapeutic application may be constrained by limited flavonoid bioavailability. Nanoparticle formulation may improve their delivery and biological activity. This study evaluated the effects of intramuscular nanoparticle-formulated QLCs on clinical and structural recovery following experimentally modified ischemic-limb compression in rabbits and estimated their pharmacokinetic withdrawal period.

Materials and Methods: Twenty adult male rabbits were allocated to four groups (n = 5/group). Macro-level tissue compression was induced by external circumferential stainless-steel ligation of the right trochanteric region for 2 weeks. Following ligature removal, the positive control group (Group A) received dexamethasone (2 mg/kg body weight), the negative control group (Group B) received sterile water, and two groups received nanoparticle-formulated QLCs (50 mg/kg body weight) intramuscularly twice daily for 5 days. One QLC-treated group (Group C) was dedicated to pharmacokinetic profiling and withdrawal time estimation using high-performance liquid chromatography, whereas the second QLC-treated group (Group D) was evaluated for post-treatment clinical recovery (lameness, swelling, and nerve reflexes) and vascular histopathology. Group comparisons were performed using the Kruskal–Wallis test ($p < 0.05$).

Results: Nanoparticle-formulated QLCs promoted greater clinical recovery at 5 days post-treatment compared with dexamethasone and sterile water. QLC-treated rabbits exhibited reduced lameness and swelling alongside greater histopathological vessel-lumen patency ($p < 0.05$). Histopathological observations confirmed better preservation of endothelial architecture and reduced structural luminal collapse in QLC-treated tissues compared to control groups; however, functional restoration of blood flow was not directly quantified. Pharmacokinetic analysis supported an estimated withdrawal interval of approximately 9–10 days. The observed vascular effects were consistent with the proposed endothelial-protective and vasodilatory actions of the QLC formulation.

Conclusion: Intramuscular nanoparticle-formulated QLCs from *D. pentandra* promoted functional and structural microvascular recovery following experimentally induced venous constriction in rabbits and performed favorably relative to dexamethasone in the measured outcomes. The estimated 9–10-day withdrawal interval provides preliminary information relevant to residue management. These findings support further investigation of nanoparticle-formulated QLCs as a plant-derived candidate for veterinary vascular repair; however, larger studies incorporating quantitative vascular perfusion measurements and comprehensive safety assessments are required before clinical application.

Keywords: *Dendrophthoe pentandra*, endothelial protection, Health and Well-Being, nanoparticle formulation, quercetin-like compounds, rabbit model, vascular recovery, venous constriction, withdrawal period.

Corresponding Author: Mochamad Lazuardi

E-mail: lazuardi@fkh.unair.ac.id

Received: 15-06-2026, **Accepted:** 23-09-2026, **Published online:** 24-10-2026

Co-authors: HP: hani-p@fkh.unair.ac.id; EPH: eka-p-h@fkh.unair.ac.id; CHC: chdvmm@asia.edu.tw; JLH: jielonghe@asia.edu.tw; QKA: Qonita.Anjani@qub.ac.uk

How to cite: Lazuardi M, Plumeriastuti H, Hestianah EP, Chien CH, He JL, Anjani QK. Nanoparticle-formulated quercetin-like compounds from *Dendrophthoe pentandra* promote vascular recovery in a rabbit model of experimentally induced venous constriction. Vet World, 2026, 19(10): 4767–4787.

Copyright: Lazuardi et al. This article is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>).



INTRODUCTION

Quercetin-like compounds (QLCs) are flavonoids isolated from *Dendrophthoe pentandra* (L.) Miq., a parasitic mistletoe locally known as *benalu duku* (BD), which commonly infests *Lansium domesticum* host trees. *D. pentandra* propagates naturally through avian seed dispersal and can also be propagated artificially through grafting onto host plants [1–3]. It is predominantly distributed in tropical and subtropical regions, particularly China, Southeast Asia, northern Australia, New Guinea, and northern New Zealand, although its distribution may extend into temperate regions depending on the availability of suitable host plants [4–6].

After extraction, BD leaves can be fractionated using methanol–ethyl acetate and n-hexane. This process yields several groups of bioactive compounds, with flavanols representing an important group of secondary metabolites. While previous literature reports a wide range of secondary metabolites in crude *D. pentandra* leaf extracts (Table 1), the test formulation evaluated in this study was specifically isolated and characterized via HPLC as a QLC subfraction (12.5% relative purity against quercetin standard).

Table 1: Secondary metabolites identified in *Dendrophthoe pentandra* mistletoe leaf extract after percolation and their reported pharmacological effects.

Compound	Pharmacological effect	References
Genistein	Androgenic and phytohormonal activity	[7, 8]
Morin	Antiproliferative and antitumor activity	[7, 9]
Kaempferol	Antioxidant, anti-inflammatory, anticancer, neuroprotective, and cardioprotective activity, including endothelial and anti-atherosclerotic effects	[7, 8]
Progesterone-like compound	Hormonal activity, including progestogenic- and androgenic-like effects	[9]
Eicosanoids	Anti-inflammatory activity	[7, 9]
Quercetin-like compounds	Antiviral, antidiabetic, anti-salmonellosis, and cell proliferation-modulating activity	[7, 8]
Pentamethoxyflavanone	Anti-inflammatory, anticancer, antioxidant, and antimitotic activity	[9]
7-hydroxy-1,2-dimethoxyxanthone	Antioxidant, anti-inflammatory, neuroprotective, and anticancer activity	[9]
Pelargonidin-3-glucoside	Antioxidant, anti-inflammatory, and anticancer activity	[7, 9]
Phenolic compounds	Antibacterial, antiviral, and antiparasitic activity	
Scutellarin	Neuroprotective, cerebrovascular protective, antioxidant, anti-inflammatory, antithrombotic, and endothelial-protective effects	[7, 8]
Tanshinol	Antioxidant, anti-inflammatory, vasodilatory, cardioprotective, antithrombotic, and neuroprotective effects	[7, 8]
Tyramine	Sympathomimetic effects, including vasoconstriction, increased blood pressure, and tachycardia	[9]
Phyto-menadione	Coagulation-related activity, including hemostatic and vascular effects	[9]
6-Aldehydoisoophiopogonone	Cytotoxic and antioxidant activity	[7–9]
Euxanthone	Antioxidant, antimicrobial, anticancer, neuroprotective, and anti-inflammatory activity	[9]
Pedalitin	Endothelial function modulation and vascular protective activity	[7–9]

Efforts to use medicinal plants as sources of therapeutic compounds align with broader initiatives to promote health and well-being, including the Sustainable Development Goals supported by the World Health Organization [10]. Plant-derived active pharmaceutical ingredients have been investigated as potential therapeutic agents because of their diverse biological activities [11, 12]. However, their safety, metabolic tolerability, and toxicological profiles require dedicated compound-specific evaluation before clinical translation. Furthermore, these compounds do not cause excessive side effects and possess a physicochemical structure that is not easily affected by chemical groups within the body. Reported activities include stimulation of skin-cell regeneration, hypoglycemic effects, modulation of androgenic activity, antibacterial activity against *Salmonella* species, and antiviral activity against Newcastle disease virus [7, 13]. These potential applications support continued investigation of plant-derived compounds for health and well-being [14, 15] and encourage further exploration of plant-based medicinal agents [16]. Reported biological activities include maintaining endothelial integrity, modulating arterial and venous constriction, inhibiting thrombotic processes associated with vascular dysfunction, promoting vascular relaxation, and exerting anti-inflammatory and antioxidant effects [7, 17]. However, the therapeutic application of plant-derived flavonoids may be influenced by their physicochemical characteristics and bioavailability. Nanoparticle formulation has been proposed to improve the delivery, liposolubility, and bioavailability of these compounds [18–20], potentially facilitating distribution of active constituents into target tissues. Nanoparticle-based formulation may also be advantageous when the yield of

individual SMCs is relatively low, reportedly approximately 5%-10% [16, 18]. In addition, the renewable availability of medicinal plants and the physicochemical stability of selected plant-derived compounds may facilitate their continued investigation as sources of therapeutic agents [21, 22]. Ethnopharmacological approaches may therefore help identify and develop new plant-derived medicines [23, 24].

The QLC constituents with reported vascular protective or vascular-modulating properties include kaempferol, morin, scutellarin, pentamethoxyflavanone, tanshinol, tyramine, phyto-menadione, and pedalitin. These compounds represent a substantial proportion of the SMCs identified within the QLC preparation. Isolation and purification of these constituents may facilitate further evaluation of their potential therapeutic applications [25–29]. Another SMCs subfraction that can be found and isolated from *D. pentandra* is quercetagin (3,3',4',5,6,7-hexahydroxyflavone) with an amount of around 0.5–1%. Quercetagin is often difficult to isolate because of its more polar physicochemical properties [17, 20].

Despite reported pharmacological activities of QLC constituents, evidence on their ability to restore vascular structure and function after sustained mechanical venous constriction remains limited. In particular, QLCs derived from *D. pentandra* growing on *L. domesticum* have not previously been formulated as nanoparticles and evaluated in a controlled experimental model of prolonged venous compression. Consequently, it remains unclear whether nanoparticle-formulated QLCs can help restore the venous lumen and vascular wall after pressure-induced constriction. In addition, data on the persistence and elimination of these compounds after administration are needed to support their potential veterinary application, particularly when treated animals may subsequently enter the food chain. Therefore, evaluating both vascular recovery and the withdrawal period is necessary to provide preliminary evidence on therapeutic performance and residue-related considerations.

This study aimed to evaluate the restorative effects of nanoparticle-formulated QLCs derived from *D. pentandra* growing on *L. domesticum* on veins subjected to experimentally induced prolonged pressure and consequent narrowing of the venous lumen. The study assessed whether QLC administration could restore the venous lumen and vascular structures after experimentally induced constriction. Rabbits were used as the experimental model to investigate clinical and histological vascular recovery. The study also aimed to determine the withdrawal period after QLC administration to provide preliminary information on residue management and appropriate slaughter timing in food-producing animals. We hypothesized that administration of nanoparticle-formulated QLCs would promote recovery of the venous lumen following experimentally induced narrowing. To evaluate the therapeutic efficacy of nanoparticle-formulated QLCs, a corticosteroid was selected as the reference positive control owing to its established role in reducing ischemia-induced vascular permeability, acute inflammatory edema, and microvascular collapse. Comparing QLC treatment against corticosteroid-anti-inflammatory enables a direct assessment of its capacity to restore vessel-lumen patency and facilitate functional limb recovery relative to a potent synthetic anti-inflammatory standard.

MATERIALS AND METHODS

Ethical approval

All animal procedures were reviewed and approved by the Ethical Review Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Animal Care and Use Committee (ACUC) (Approval No. 1.KEH.12.02.2025; February 6, 2025). Ethical approval preceded all live-animal experimentation, which commenced on February 15, 2025. The study was conducted in accordance with the modified Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 guidelines following assessment by the Animal Ethics Committee. Animal health and physiological conditions were monitored and certified by the ACUC.

The study also followed international guidelines and recommendations for animal care and use, including the Organization for Economic Co-operation and Development (OECD) Guidance Document No. 19 on Humane Endpoints in Animal Care and Use. These guidelines emphasize the fundamental 3R principles of Replacement, Reduction, and Refinement, including minimizing the number of experimental animals while maintaining an appropriate and comparable number of animals required to obtain reliable research outcomes.

To safeguard animal welfare, predefined humane endpoints were established in accordance with OECD Guidance Document No. 19. Rabbits meeting any of the following criteria were immediately removed from the study and humanely euthanized: (1) rapid, unexplained body weight loss exceeding 20% of baseline body weight over a 3-day period; (2) severe pain or distress characterized by unresponsiveness to standard analgesia, persistent vocalization, complete absence of grooming, or prolonged recumbency exceeding 24 h; (3) severe

localized ischemia resulting in extensive ulceration, dry or wet gangrene extending beyond the ligature line, or systemic sepsis characterized by hypothermia (<37°C) or severe lethargy; and (4) physiological compromise characterized by persistent anorexia, defined as complete absence of eating or drinking for >36 h, or severe motor dysfunction preventing the animal from reaching food or water.

Study period and location

The study was conducted from early September 2024 to mid-April 2026 at several laboratories associated with the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, particularly the Research Center and Applied of Indonesia Veterinary Pharmacy and Pharmacology Association. The procedures used for QLC isolation and nanoparticle production were deposited in a data repository. Non-animal phytochemical isolation was conducted from September 2024 to January 2025; live-animal work commenced Feb 15, 2025, following ACUC approval.

Study design

The study used a post-test-only controlled experimental design. Adult male rabbits (*Oryctolagus cuniculus domesticus*) weighing 2.5–2.6 kg (mean ± standard deviation: 2.58±0.045kg; %CV = 1.733%) were obtained from a commercial breeder. Rabbits serve as an established translational model for microvascular and ischemic tissue injury because their vascular architecture is well characterized and their structural vessel responses are comparable to the human microvasculature [26]. The animals were randomly allocated into four groups (n=5/group) using a simple randomized lottery draw based on individual identification numbers: positive control (Group A), negative control (Group B), pharmacokinetics group (Group C), and post-treatment clinical and histopathological recovery evaluation group (Group D). Each rabbit was the experimental unit for all statistical, pharmacokinetic, clinical, and histopathological analyses.

The required number of rabbits (N) was calculated using Equation (1):

$$N = \frac{\left[\left\{ Z_{1-\left(\frac{\alpha}{2}\right)} + Z_{\beta} \right\}^2 \right]}{\left\{ \frac{(d)^2}{(S_a)^2 + (S_b)^2} \right\}} \text{ Equation (1)}$$

At a significance level of 0.05 and a 5% margin of error, the following parameters were used: $Z_{1-(\alpha/2)} = 1.96$, $Z_{\beta} = 1.645$, $d = 3.62$, $S_a = 1.7$, and $S_b = 1.4$. Z_{β} was determined based on populations with equal sample sizes [32]. S_a and S_b were calculated for the two populations with equal sample sizes [33]. Equation (1) yielded $N = 4.88$ rabbits, which was rounded to five rabbits.

All experimental groups underwent a modified ischemic-limb compression model using external circumferential ligation of the upper right trochanteric region for 2 weeks. This macro-level compression model was established using stainless-steel wire (2.5–3.0 mm diameter, 10 cm length) placed 0.5–0.7 cm distal to the superior thigh base to induce downstream blood flow restriction and tissue ischemia, rather than selective or direct vascular ligation. Compression was standardized to a binding loop diameter of approximately 0.75–1.0 cm (measured with calipers). Sterile cotton padding was placed on the skin laceration. Local analgesia (lidocaine 0.03 mg/kg BW SC every 12 h for 48 h) and antiseptic care (0.5% chlorhexidine) were maintained. Constriction was confirmed clinically prior to treatment by evaluating swelling, reflex impairment, and lameness. (Figure 1) [34, 35]. The experimental animals were physically healthy, and health examinations were performed every 12 h after ligation. After 2 weeks of ligation, the ligature was removed, followed by treatment administration.

Following ligature removal, control groups (A and B) and the trial group (C and D) received their respective treatments twice daily (every 12 h) for 5 consecutive days (10 total injections). Clinical monitoring was performed every 12 h, with final endpoint clinical scoring recorded at the end of day 5 (12 h post-final dose). Following a standardized 45-minute interval post-assessment, Groups A, B, and D underwent euthanasia and tissue collection at equivalent time points. Group A received dexamethasone at 2 mg/kg BW. Dexamethasone was selected as the positive control due to its established anti-inflammatory and anti-edematous efficacy in compression and ischemia-reperfusion models, serving as a clinical and histological reference standard to evaluate the non-steroidal vascular-recovery effects of QLCs. Group B received 1 mL sterile water for parenteral injection (PT Otsuka, Pasuruan, East Java, Indonesia). Group C underwent serial blood sampling for pharmacokinetic profiling, while Group D underwent clinical recovery scoring (lameness, swelling, and nerve reflexes) and vascular histopathology. The clinical condition was evaluated in a blinded manner by 3 veterinary assessors with no conflicts of interest with the research team. A schematic overview of the experimental procedure is presented in Figure 2.



Figure 1: Right trochanteric ligation in rabbits.

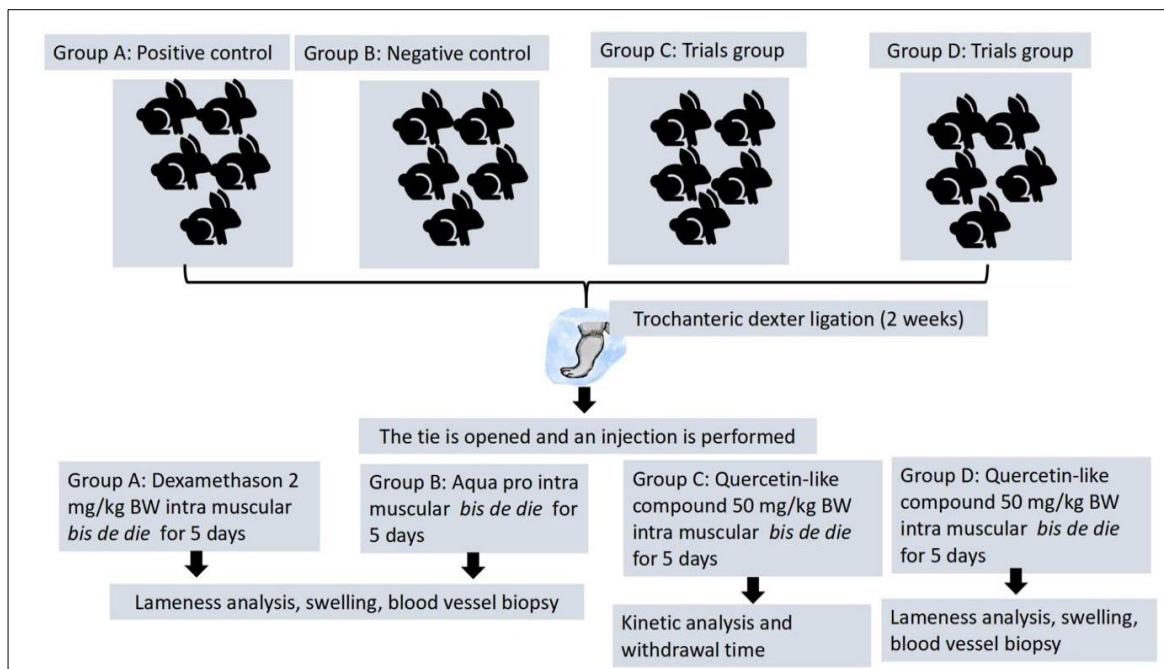


Figure 2: Animal model for the analysis of quercetin-like compounds as venous vasodilation stimulants.

Preparation of the sterile injectable formulations

The preparation was performed aseptically in a vertical laminar-flow cabinet (1300 Series A2, Thermo Fisher Scientific, Suzhou, China). A total of 1 g of dried QLC nanoparticles was dissolved in 10 mL of dimethyl sulfoxide (DMSO; 10% w/v) to prepare a solution. The solution was subsequently filtered through a 20- μ m filter and used to prepare the injectable formulation for administration to the experimental animals.

Following nanoparticle formation, DMSO (catalog no. D2650; Merck KGaA, Darmstadt, Germany) was used at a 1:2 ratio as the solvent. The pH was adjusted to 6.6-7.0 by adding 1-2 drops of 0.1 N hydrochloric acid. Subsequently, sterile water for injection (Kimia Farma, Indonesia) was added as a diluent for the injectable preparation. The final injectable formulation had a QLC concentration of 100 mg/mL, corresponding to an administered injection volume of 1.25–1.30 mL per dose (50 mg/kg BW). Formulation quality was confirmed prior to administration: sterility was verified per USP <71> using membrane filtration with a 14-day incubation in Fluid Thioglycollate and Soybean-Casein Digest Media showing no microbial growth; apyrogenicity was established per USP <85> via the Limulus Amebocyte Lysate gel-clot assay (<0.25 EU/mL); and physicochemical parameters were maintained at pH 6.8 ± 0.1 and an osmolality of 290 ± 10 mOsm/kg [30, 31].

Clinical monitoring and daily care of experimental animals

All experimental rabbits were housed individually in temperature-controlled rooms at $25 \pm 1^\circ\text{C}$ and 20% relative humidity under a 12-h light/dark cycle, with free access to standard feed and water. During the 14-day trochanteric ligation period, a trained veterinarian monitored the animals twice daily at 12-h intervals.

Monitoring included general clinical health, food and water consumption, body weight, local skin integrity at the ligation site, and behavioral signs of acute pain or distress.

Analgesia and infection control

To minimize procedure-related pain without interfering with the non-genomic inflammatory and

vasodilatory endpoints of QLCs, lidocaine (0.03 mg/kg, subcutaneously) was administered preemptively before ligation and subsequently every 12 h for the first 48 h after the procedure.

Antiseptic care of the ligated right trochanteric region was performed daily using 0.5% chlorhexidine gluconate solution to prevent localized bacterial infection and tissue necrosis.

Blood collection

Blood samples (1 mL) were collected from Group C rabbits relative to the final (10th) dose on Day 5 following repeated twice-daily intramuscular administration (50 mg/kg BW). Time-zero ($t = 0$ min) was designated as the trough sample drawn immediately prior to the 10th injection, representing residual systemic accumulation (C_{trough}) from preceding doses. Raw plasma concentrations were reported and analyzed without baseline subtraction or mathematical correction to reflect true steady-state exposure. Serial blood collection followed at 5, 10, 20, 40, 60 and 80 min post-injection (accounting for a 5-minute initial post-dose lag time). Blood samples were collected in 3 mL tubes containing heparin, and whole blood samples were mixed with heparin. Then, they were centrifuged at 1,500 rpm for 30 minutes to obtain plasma. The plasma was then placed in special vials for further preparation and stored in a -80°C freezer until the blood samples were prepared for HPLC analysis.

Sample preparation

Blood samples were mixed with 1 mL of high-performance liquid chromatography (HPLC)-grade methanol and shaken vigorously. The samples were subsequently vortexed using a Vortex Genie and centrifuged at $865 \times g$ for 10 min using an EBA 200 centrifuge (Andreas Hettich GmbH & Co.) to obtain the supernatant.

The supernatant was dried under nitrogen gas using a water bath maintained at 37°C . The dried supernatant was reconstituted in 1 mL of HPLC-grade methanol:HPLC-grade water (40:60) and subsequently applied to a solid-phase extraction octadecyl xylene (SPE ODS) cartridge (catalog no. 52602-U; Merck Corp., USA).

Before sample application, the SPE ODS cartridge was activated by successive washing with methanol, followed by methanol:water (40:60), and then water. The resulting eluate was filtered through a $20\text{-}\mu\text{m}$ filter (catalog no. SLFG025LS; Merck Corp., USA) and was subsequently used for HPLC analysis to determine QLC concentrations.

After drug administration, rabbits in Groups A, B, and D were euthanized, and specimens comprising the right femoral blood vessels were collected for histopathological examination.

Analysis of recovery following ligation and biopsy of the right trochanteric blood vessels

Following ligation of the right trochanteric region, the ligature was removed, and the animals were evaluated to determine whether the administered analyte exhibited vasodilatory effects on the blood vessels. Findings from the treated right limb were compared with those from the contralateral left thigh of each rabbit.

The evaluation comprised three grades based on standardized semiquantitative behavioral and physical scoring criteria adapted from established microvascular and ischemic-injury literature [1, 2, 36, 37]:

- Grade A: Lameness severity was assessed as Grade 1 (mild), Grade 2 (moderate), or Grade 3 (severe).
- Grade B: Swelling characterized by erythema, increased warmth, and pain was assessed as Grade 1 (mild), Grade 2 (moderate), or Grade 3 (severe).
- Grade C: Right-leg nerve reflexes were assessed as Grade 1 (mild), Grade 2 (moderate), or Grade 3 (severe).

Clinical evaluations of lameness, swelling, and nerve reflexes were conducted independently by a blinded clinician. Interobserver agreement method was evaluated using Fleiss' kappa coefficient (κ) at <0.001 (repository).

Euthanasia and collection of vascular specimens

To safeguard animal welfare, predefined humane endpoints were established in accordance with OECD. Rabbits meeting any of the following criteria were immediately removed from the study and humanely euthanized:

- Body Weight Loss: Rapid, unexplainable weight loss exceeding 20% of baseline body weight over a 3-day period.
- Severe Pain/Distress: Unresponsiveness to standard analgesia, persistent vocalization, complete lack of grooming, or prolonged recumbency exceeding 24 hours.
- Tissue Necrosis & Infection: Severe localized ischemia resulting in extensive ulceration, dry/wet gangrene extending beyond the ligature line, or systemic sepsis (hypothermia $< 37^{\circ}\text{C}$ or severe lethargy).
- Physiological Compromise: Persistent anorexia (complete absence of eating or drinking for > 36 hours) or severe motor dysfunction rendering the animal unable to reach food/water.

Further analysis involved blood-vessel biopsy. Rabbits in Groups A, B, and D were first anesthetized with ketamine at 50 mg/kg BW, administered intramuscularly into the right hindlimb. Euthanasia was subsequently performed by intravenous administration of sodium pentobarbital at 100 mg/kg BW through the marginal ear vein while the animals were under deep anesthesia.

Following euthanasia, an incision was made to collect blood vessels from the right thigh. The specimens were placed in glass containers containing 40% (v/v) formalin for histopathological examination.

Histopathological examination

Specimens were stained with hematoxylin and eosin and evaluated at 200× magnification [38]. Histopathological examination was conducted on tissue sections collected from the right trochanteric femoral vessel region. For each rabbit (n = 5 per group), five (5) randomly selected high-power fields (HPFs) were systematically evaluated under light microscopy at 200x magnification by three blinded pathologists. Across each experimental group, a total of 25 HPFs were blindly evaluated. Microvessels within each field were evaluated based on predefined structural criteria:

- Vasodilation: Defined as a fully patent, open vascular lumen with relaxed smooth muscle walls, intact endothelial architecture, and an unhindered cross-sectional lumen free of mechanical collapse or thrombi.
- Vessel Occlusion: Defined as structural luminal collapse, significant mechanical narrowing (>50% reduction relative to normal vessel caliber), microvascular thrombosis, or luminal obstruction characterized by packed erythrocytes and fibrin deposition.

To aggregate findings into the animal-level outcomes reported in Table 6, each rabbit was categorized according to its predominant vascular phenotype across all 5 examined HPFs. An animal was designated as exhibiting Vasodilation if >50% of evaluated microvessels met the criteria for luminal patency and relaxation, or as Vessel Occlusion if >50 of vessels displayed luminal collapse or thrombosis. Group-level ratios (e.g., 5/5 or 0/5) reflect the proportion of rabbits in each group displaying the primary phenotype. It should be noted that histopathological scoring evaluated structural parameters (luminal patency, endothelial wall integrity, and absence of mechanical collapse or luminal thrombosis). These measurements represent tissue-level morphological observations and do not constitute direct functional measurements of blood flow or tissue perfusion. To minimize assessment bias, histopathological evaluations were performed using a blinded protocol. Two people conducted the initial examination: the attending veterinary pathologist at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, and a master's scholar in veterinary medicine. The pathology and histology team subsequently cross-examined and verified the findings by consensus. The evaluated parameters were systematically analyzed using Fleiss' kappa (κ) coefficient, following the same inter-rater agreement protocol applied during the clinical recovery assessment.

Optimization and validation of QLC analysis using preparative HPLC

The selectivity criterion (α) was defined as the separation between the peak area of the QLC sample or quercetin standard and any interfering or impurity peak. The selectivity factor was required to differ from 1 ($\alpha \neq 1$).

Method validation was performed using a blank rabbit plasma matrix and included four parameters: (1) linearity, (2) intra-day precision, (3) recovery (%), and (4) sensitivity, including the limit of detection (LOD) and limit of quantification (LOQ). Rabbit plasma was obtained from animals of the same species described in the study design and prepared before analysis [39].

Linearity and intra-day precision were evaluated using quercetin concentrations ranging from 0.01 to 1.00 $\mu\text{g/mL}$ in a rabbit plasma matrix [40]. The procedure was repeated five times, and the mean of the five resulting peak areas was used as the final value.

A series of QLC standards at concentrations of 0.01, 0.15, 0.25, 0.50, 0.75, and 1.00 $\mu\text{g/mL}$ was prepared by dissolving the compounds in HPLC-grade methanol (w/v). These standards were injected into the HPLC system using the preset analytical parameters. The resulting peak areas were used to construct a calibration curve, with concentration ($\mu\text{g/mL}$) plotted on the X-axis and peak area on the Y-axis.

Subsequently, quercetin concentrations ranging from 0.01 to 1.00 $\mu\text{g/mL}$ were prepared in a rabbit plasma matrix. Quercetin concentrations in these samples were determined using the previously described sample preparation method. Results from the linearity and intra-day precision analyses were used to determine the coefficient of determination (R^2) and calculate the percentage coefficient of variation.

Accuracy was evaluated by percentage recovery using quercetin concentrations ranging from 0.01 to 1.00

µg/mL in rabbit plasma. Sample preparation involved extraction of quercetin from plasma into methanol to determine its concentration. The analysis was performed five times.

Sensitivity, LOD, and LOQ

A sensitivity test was performed to establish the LOD and LOQ based on the standard deviation of the response and the slope. Initially, the lowest concentration that continued to produce a detectable quercetin chromatographic peak in the plasma matrix was determined using three concentrations, and the relationship $Y = A + BX$ was established.

Standard quercetin was subsequently spiked into artificial rabbit plasma following the procedure described in the sample preparation section. Peak area (Y) was plotted against standard quercetin concentration (X). The slope (S) obtained from the linear equation was used to determine the LOD and LOQ, whereas the standard error of estimation (SEE) was calculated from the regression equation.

The LOD value was $(3.3 \times \text{SEE})$, while the LOQ value was $(10 \times \text{SEE}) / S$.

The minimum detectable quercetin concentration was further confirmed using a signal-to-noise ratio of approximately 5:1 between the analyte peak and baseline noise or interfering peaks.

Determination of QLC levels in plasma and kinetic profile analysis

Samples from Group C were analyzed according to the following procedure. First, a standard curve was prepared using standard concentrations of 0.10, 0.25, 0.50, 0.75, and 1.00 µg/mL in artificial rabbit plasma.

Samples prepared in rabbit plasma were transferred to clean HPLC vials, filtered through a 0.20-µm filter, and injected into the HPLC system to obtain peak areas used to determine concentrations. For the prepared analyte samples, 3 mL of mobile-phase eluent was added to clean HPLC injector vials, shaken for 10 min, filtered through a 0.20-µm filter, and subsequently injected into the HPLC system.

Pharmacokinetic characterization of QLCs was performed using non-linear least-squares regression. One-compartment, two-compartment, and non-compartmental open kinetic models were systematically evaluated. The optimal model was selected based on goodness-of-fit criteria, including the Akaike Information Criterion (AIC) and the coefficient of determination (R^2).

The pharmacokinetic parameters included the drug distribution rate constant (α , min^{-1}), drug absorption rate (K_a), and drug elimination rate (β). Additional parameters included the time required to reach maximum concentration (T_{max} , min) and maximum concentration (C_{max} , µg/mL). Further parameters included the area under the QLC concentration-time curve (AUC, µg/mL·min), central distribution volume (V_d central, mL), clearance (Cl, mL/min), and mean residence time (MRT, min), representing the average period for which QLCs remained in the rabbit's body.

Determination of withdrawal time

The withdrawal time of QLCs in rabbits was determined using Equation (2) based on the pharmacokinetic parameters obtained [41]. The elimination half-life ($T_{1/2\beta}$) was calculated as $0.693/\beta$, where β represents the elimination rate constant (min^{-1}). The accumulation factor (R) was set at 1.306.

Other variables included the total administered dose (mg), LOQ (Clim , µg/mL), and safety factor (SF), which ranged from 1 to >1 . The SF was set at 1 when a country's food-safety regulations were considered fully effective and at >1 when regulatory controls were assumed to be less stringent.

For calculation of the QLC withdrawal time, the administered dose was 50 mg/kg body weight twice daily (*bis in die*) for 5 days. The results indicated that the animals were expected to be free of QLCs approximately 10 days after therapy. The withdrawal time for Group C was determined as follows [42, 43]:

$$\text{Withdrawal time} = \frac{T_{1/2\beta} \times (\ln R \times \text{Dose} - \ln \text{Clim})}{\ln 2} \times \text{SF} = (\text{times}) \text{ Equation (2)}$$

Statistical analysis

Outcomes in the control groups (Groups A and B) and experimental group (Group D) were compared using the Kruskal–Wallis test followed by pairwise post hoc comparisons. For comparison, outcomes from the two control groups were pooled and compared with those from Group D, including recovery observations classified as Grades A, B, and C after administration of dexamethasone and sterile water for injection.

Similarly, histopathological outcomes, including vascular occlusion and vasodilation, were compared between the control groups and Group D. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS software version 27 (IBM SPSS, Chicago, USA).

RESULTS

Characterization of QLCs

QLCs isolated from a subfraction of *D. pentandra* leaf extract were characterized using HPLC. The isolated fraction showed distinct retention times of 8.263 min at $\lambda = 250.4$ nm and 8.366 min at $\lambda = 255.4$ nm, matching the certified quercetin reference standard. Based on the deposited repository data (DOI: 10.6084/m9.figshare.31112728), the purified QLC fraction had a relative constituent purity of 12.5% (w/v) against quercetin and a residual solvent content of 1.2% (w/v).

The experimental design involving artificial compression (Figures 1 and 2) provided an animal model for investigating venous occlusion and subsequent recovery. Rabbits enabled monitoring of the recovery response after ligature removal.

Nanoparticle characterization

The results of nanoparticle fabrication are presented in Figure 3. Before Diethylaminoethyl (DEAE) column separation (prior to final nanoparticle formation), particle sizes ranged from 18.23 to 2015.34 nm (Figure 3A), whereas after column separation, particle sizes ranged from 14.92 to 149.25 nm (Figure 3B). DEAE-assisted cavities contributed to generating very small, relatively uniform particles. This separation strategy was originally developed for isolating trypanosome parasites from the bloodstream of experimental rats, with dimensions of $14\text{--}33\text{ }\mu\text{m} \times 1.5\text{--}3.5\text{ }\mu\text{m}$ [44], and its application to nanoparticle processing represents an adaptation of this technique. Although DEAE anion-exchange matrix separation yielded refined nanoscale particles, residual DEAE levels and their potential biological effects were not directly quantified in this study. DEAE-assisted separation was utilized as an anion-exchange chromatographic matrix to generate small, relatively uniform particles. Within this preparation method, the most frequently observed particle size range was 40.62–54.84 nm (Figure 4), with a cumulative particle count averaging approximately 11%–13%. The relationship between particle diameter and SMC QLC availability ranged from 0.01% at 16.50 nm to 13.87% at 49.62 nm. The cumulative particle size distribution thresholds were 32.29 nm for D_{10} , 46.84 nm for D_{50} , and 67.67 nm for D_{90} .

Zeta potential and polydispersity index (PDI) were assessed qualitatively by monitoring formulation stability and dispersion uniformity *in vivo*, rather than through direct physicochemical quantification. Nanoparticle synthesis involved ultrasonic deconstruction at 40 kHz for 2 h at 40°C. Although ultrasonic deconstruction exhibited batch-to-batch size dispersion, with a relative standard deviation (percent coefficient of variation, %CV) of 44.29%–58.16% for the median particle diameter at 46.84 nm (D_{50}) across synthesis runs relative to the target 100 nm nanoscale threshold, the resulting particle sizes predominantly met the nanoscale criterion (<100 nm). Furthermore, physical synthesis combined with DEAE filtration produced a D_{90} value below 70 nm (67.67 nm), confirming that the vast majority of the formulation maintained nanoscale dimensions (<100nm). The resulting small particle size was considered potentially favorable for QLC distribution within biological tissues [45].

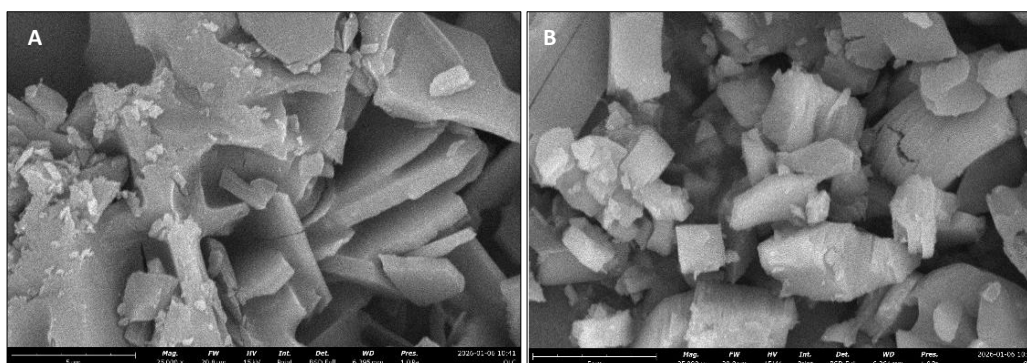


Figure 3: Particle size distribution of analytes: (A) before DEAE column separation (prior to nanoparticle formation), ranging from 18.23 to 2015.34 nm, and (B) after DEAE column separation (following nanoparticle formation), ranging from 14.92 to 149.25 nm.

HPLC method validation

The results of the linearity, intra-day precision, accuracy, and recovery analyses are presented in Table 2. These analyses were performed by the same operator using established procedures [43]. Table 3 summarizes the validation parameters evaluated during system-suitability testing to verify analytical reliability. The method was

evaluated for key parameters, including selectivity, linearity, intra-day precision, accuracy, LOD, and LOQ, in alignment with the validation principles outlined in ICH guidelines [42, 43].

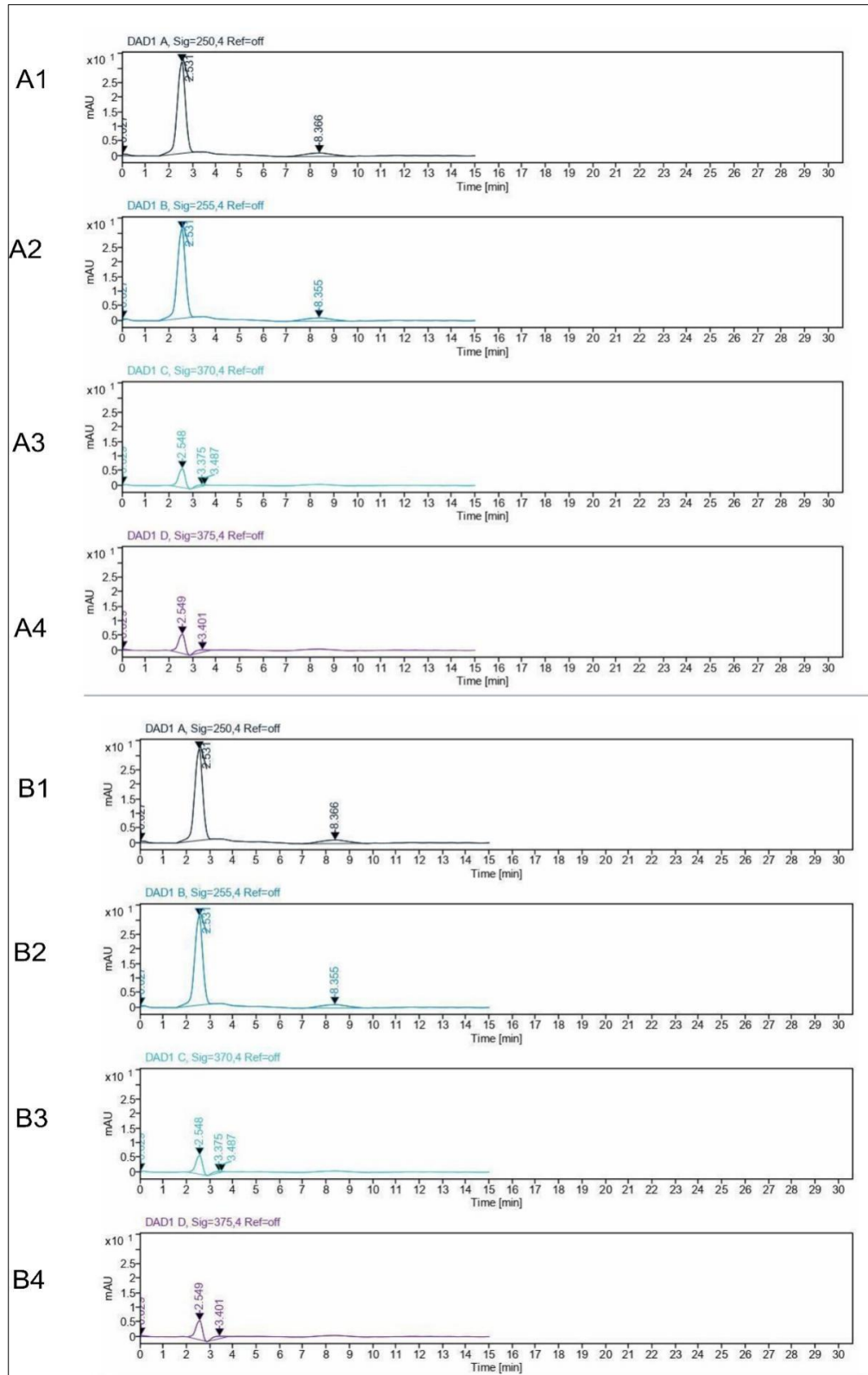


Figure 4: (A1-A4) Chromatogram peak areas of quercetin-like compounds (QLCs) at 0.150 $\mu\text{g/mL}$ in methanol (w/v) obtained using a high-performance liquid chromatography system (Agilent 1260). (B1–B4) Chromatogram peak areas of QLCs at 0.138 $\mu\text{g/mL}$ in rabbit plasma (w/v).

Sensitivity analysis showed that the lowest concentration producing a detectable peak area was 1×10^{-2} $\mu\text{g/mL}$ (0.01 $\mu\text{g/mL}$), with a peak area of 21.16942. Subsequent analyses used two higher concentrations: 2×10^{-2} $\mu\text{g/mL}$ (peak area 44.33880) and 3×10^{-2} $\mu\text{g/mL}$ (peak area 62.51826). These empirical values demonstrate close agreement with the calculated limit of detection ($\text{LOD} = 8.6 \times 10^{-3}$ $\mu\text{g/mL}$) and limit of summarized in Table 3. The

resulting linear equation was $Y = 138.1X + 21.0$ ($R^2 = 0.995$), from which S was determined to be 138.1 and SEE was 0.36. This low-range calibration curve ($0.01=0.03 \mu\text{g/mL}$) was evaluated specifically for LOD/LOQ estimation, whereas the full-range linearity curve ($0.01-1.00 \mu\text{g/mL}$) showed an R^2 of 0.998, as presented in Table 3. Sensitivity parameters based on this calculation are summarized in Table 3. Figure 4 shows the peak areas of the QLC standard in methanol and SMC analytes in rabbit plasma.

A1-A4 represent the peak areas of QLCs at $0.150 \mu\text{g/mL}$ in methanol. Peak areas were observed at a retention time of 8.263 min at 250.4 nm (A1) and 8.366 min at 255.4 nm (A2). No peak area was detected at 370.4 nm (A3) or 375.4 nm (A4). B1-B4 represent the peak areas of QLCs at $0.138 \mu\text{g/mL}$ in rabbit plasma. Peak areas were observed at a retention time of 8.366 min at 250.4 nm (B1) and 8.366 min at 255.4 nm (B2). No peak area was detected at 370.4 nm (B3) or 375.4 nm (B4).

Table 2: Intra-day precision and accuracy parameters based on recovery of quercetin standards in rabbit plasma over five replicates.

Calculated concentration ($\mu\text{g/mL}$)	Peak area	Peak area, $\bar{x} \pm \text{SD}$	Concentration ($\mu\text{g/mL}$)	Intra-day precision, $\bar{x} \pm \%CV$	Recovery (%)	Accuracy, $\bar{x} \pm \text{SD}$
0.010	206.51538	207.115314 ± 0.894	0.012	0.011 ± 7.470	120.000	112 ± 8.367
	206.51522		0.011		110.000	
	206.51533		0.010		100.000	
	207.51543		0.012		120.000	
	208.51521		0.011		110.000	
0.150	371.78154	370.974988 ± 0.832	0.142	0.135 ± 6.412	94.666	89.866 ± 5.762
	370.78821		0.138		92.000	
	369.79185		0.121		80.666	
	370.74180		0.132		88.000	
	371.77154		0.141		94.000	
0.250	743.48242	742.861998 ± 2.255	0.281	0.273 ± 5.722	112.400	109.04 ± 6.239
	742.44441		0.272		108.800	
	741.46311		0.261		104.400	
	746.38443		0.294		117.600	
	740.53562		0.255		102.000	
0.50	1306.46484	1336.421996 ± 33.659	0.464	0.496 ± 3.777	92.800	99.16 ± 3.745
	1362.95191		0.508		101.600	
	1313.17111		0.497		99.400	
	1317.87101		0.499		99.800	
	1381.65111		0.511		102.200	
0.75	21248.00005	21381.97334 ± 135.389	0.741	0.754 ± 1.199	98.800	100.506 ± 1.205
	21567.16655		0.763		101.733	
	21264.16653		0.751		100.133	
	21465.16644		0.762		101.600	
	21365.36714		0.752		100.266	
1.00	284430.66862	285131.4884 ± 3578.450	1.002	1.002 ± 0.130	100.200	100.300 ± 0.158
	285531.56881		1.003		100.300	
	279931.76891		1.001		100.100	
	285831.66771		1.004		100.400	
	289931.76792		1.005		100.500	

Table 3: HPLC method validation: System-suitability test results.

Parameter	Symbol/unit	Result
Selectivity	A	Acceptable (>1)
Linearity	R^2	0.998
Intra-day precision	CV (%)	0.130-7.470
Accuracy	SD	<10.00
Recovery	%	89.866-112
Sensitivity	LOD ($\mu\text{g/mL}$)	8.6×10^{-3}
Total	LOQ ($\mu\text{g/mL}$)	2.6×10^{-2}

Plasma concentration-time profile, pharmacokinetic parameters and withdrawal time

The results of QLC analysis in Group C are presented in Table 4A. Pharmacokinetic parameters were evaluated by fitting plasma concentration–time data to one-compartment, two-compartment, and non-compartmental open models using non-linear least-squares regression. A two-compartment open model was

selected as the optimal kinetic fit, demonstrating superior goodness-of-fit with the lowest Akaike Information Criterion (AIC = -12.478) and $R^2 > 0.985$, whereas a one-compartment model yielded inferior fit statistics (AIC = 6.120). The serial sampling scheme ($t = 0, 5, 10, 20, 40, 60, 80$ min) adequately supported characterization of both the rapid distribution phase ($\alpha = 0.079 \pm 0.078 \text{ min}^{-1}$) surrounding $T_{\max}$ (10 min) and the initial terminal elimination phase ($\beta = 0.027 \pm 0.007 \text{ min}^{-1}$, $T_{1/2\beta} \approx 25.7$ min). Furthermore, the estimated intramuscular absorption rate constant ($K_a = 0.184 \pm 0.032 \text{ min}^{-1}$) was higher than β , indicating rapid systemic absorption prior to elimination. The estimated first-order absorption rate constant ($K_a = 0.184 \pm 0.032 \text{ min}^{-1}$) was substantially higher than the terminal elimination rate constant ($\beta = 0.027 \pm 0.007 \text{ min}^{-1}$), demonstrating rapid systemic uptake of QLC nanoparticles following intramuscular administration.

Table 4: Plasma concentration-time profile ($\mu\text{g/mL}$) in Group C rabbits following the 10th consecutive intramuscular dose of QLC nanoparticles (50 mg/kg BW twice daily for 5 days).

A. Plasma concentration-time profile ($\mu\text{g/mL}$).

Time (min)	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Mean $\pm$ %CV
0	0.2	0.4	0.3	0.27	0.4	0.314 ± 27.388
5	0.6	0.7	0.6	0.71	0.7	0.662 ± 8.572
10	1.2	1.1	1.3	1.11	1.1	1.162 ± 6.840
20	1.1	1.0	1.1	1.7	1.0	1.180 ± 24.996
40	0.9	0.9	1.0	1.0	0.9	0.940 ± 5.827
60	0.6	0.8	0.8	0.8	0.8	0.760 ± 11.769
80	0.4	0.4	0.5	0.5	0.4	0.440 ± 12.448

B. Pharmacokinetic parameters

Parameter	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Mean $\pm$ %CV
Body weight (kg)	2.5	2.6	2.6	2.6	2.6	2.58 ± 1.733
α (min^{-1})	0.214	0.024	0.076	0.03	0.05	0.079 ± 99.319
β (min^{-1})	0.02	0.0346	0.0235	0.023	0.035	0.027 ± 25.562
$T_{\max}$ (min)	10	10	10	10	10	10.00
$C_{\max}$ ($\mu\text{g/mL}$)	1.2	1.1	1.1	1.1	1.1	1.12 ± 3.993
$\text{AUC}_{0-\infty}$ ($\mu\text{g}\cdot\text{min/mL}$)	90.204	86.811	96.527	106.789	77.18	91.502 ± 12.068
V_d (central) (mL)	12.959	10.994	14.325	30.647	9.913	15.768 ± 53.854
Clearance (mL/min)	1.318	1.424	1.436	1.158	1.602	1.389 ± 11.797
MRT (min)	42.7	34.0	52.4	51.6	46.7	45.48 ± 16.540
K_a (min^{-1})*	0.210	0.185	0.152	0.221	0.154	0.184 ± 17.082

*: K_a represents the first-order absorption rate constantly estimated via residual curve stripping.

To relate systemic exposure to the administered multi-constituent mixture, HPLC quantification of plasma QLC levels was conducted using a standardized, purified QLC reference standard known to contain approximately 60% quercetin. Concentrations were derived directly from the integrated chromatographic peak area of the reference standard compared to the target analyte in the rabbit plasma matrix (Figure 4), representing a standardized quercetin-equivalent response. Pharmacokinetic depletion modeling yielded an elimination rate constant (β) of 0.027 min^{-1} ($T_{1/2\beta} = 25.7$ min) and an accumulation factor ($\ln R$) of 0.267. Following a cumulative dosage of 1290 mg (50 mg/kg twice daily for 5 days), the projected withdrawal time required to reach the analytical limit of quantification ($C_{\text{lim}} = 0.026 \mu\text{g/mL}$) with a safety factor of 1.0 was 13,559 min (9.41 days), supporting a conservative residue depletion estimate of 9–10 days post-administration.

Clinical recovery and vascular histopathology

Analysis across 3 evaluators showed highly significant results ($\kappa < 0.001$). The results show consistent observations across evaluators. The individual observation results can be found in Table 5 (repository). Intergroup findings for Groups A, B, and D regarding recovery status and blood-vessel vasodilation following treatment are presented in Table 6.

Table 5: Individual score breakdown.

Group	Treatment	Animal Identification	Grade A (Lameness)	Grade B (Swelling)	Grade C (Nerve Reflexes)
Group A	Dexamethasone (2 mg/kg)	Rabbit A1	2 (Moderate)	2 (Moderate)	2 (Moderate)
		Rabbit A2	3 (Severe)	2 (Moderate)	3 (Severe)
		Rabbit A3	2 (Moderate)	3 (Severe)	2 (Moderate)

Group	Treatment	Animal Identification	Grade A (Lameness)	Grade B (Swelling)	Grade C (Nerve Reflexes)
Group B	Vehicle Control (Sterile Water)	Rabbit A4	3 (Severe)	3 (Severe)	2 (Moderate)
		Rabbit A5	2 (Moderate)	2 (Moderate)	3 (Severe)
		Rabbit B1	3 (Severe)	3 (Severe)	3 (Severe)
		Rabbit B2	3 (Severe)	3 (Severe)	3 (Severe)
		Rabbit B3	3 (Severe)	3 (Severe)	3 (Severe)
		Rabbit B4	3 (Severe)	3 (Severe)	3 (Severe)
Group D	QLC Nanoparticles (50 mg/kg)	Rabbit B5	3 (Severe)	3 (Severe)	3 (Severe)
		Rabbit D1	1 (Mild)	1 (Mild)	1 (Mild)
		Rabbit D2	2 (Moderate)	1 (Mild)	1 (Mild)
		Rabbit D3	1 (Mild)	2 (Moderate)	2 (Moderate)
		Rabbit D4	1 (Mild)	1 (Mild)	1 (Mild)
		Rabbit D5	2 (Moderate)	1 (Mild)	2 (Moderate)

Histopathological findings after treatment are presented in Figure 5. The square annotations in Figures 5A and 5B indicate representative trochanteric muscle microvessels from Groups A (dexamethasone) and B (vehicle control), demonstrating structural lumen collapse, narrowing, and erythrocyte packing indicative of persistent vessel occlusion (observed in 2/5 and 5/5 rabbits, respectively, as reported in Table 6). In contrast, the annotated section in Figure 5C provides a qualitative histopathological observation of a representative microvessel from Group D (QLCs), illustrating patent microvascular luminal architecture with an open, uncollapsed lumen and preserved endothelial morphology (observed in 5/5 rabbits).

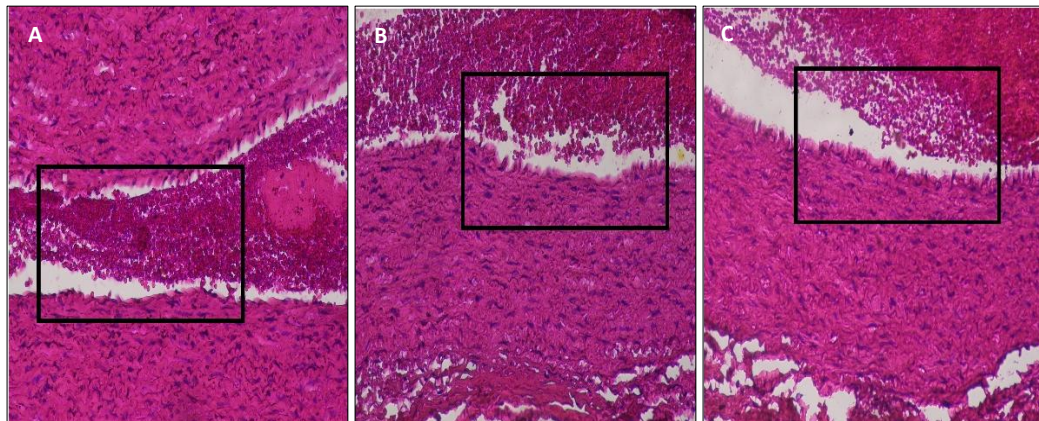


Figure 5: Representative qualitative histopathological observations of right trochanteric muscle microvessels (H&E, 200× magnification). Square annotations highlight morphological feature areas: (A) positive control group (dexamethasone) showing partial structural compression and luminal narrowing; (B) negative control group (vehicle control) showing severe structural vessel occlusion and luminal collapse; and (C) QLC-treated group showing preserved luminal architecture and open vascular morphology without evidence of structural occlusion.

Table 6: Evaluation of vascular recovery and vasodilation in the right trochanteric muscle following vessel ligation and treatment with dexamethasone, sterile water for injection (vehicle control), or quercetin-like compounds (QLCs).

Group	Treatment	Morphological Luminal Patency/Vasodilation (n/5)*	Vessel occlusion (n/5)	Grade A (lameness)	Grade B (swelling)	Grade C (reflexes)	Overall p-value	Kruskal–Wallis post hoc analysis (p-value)
Group A	Dexamethasone	3/5	2/5	Mild to severe (2-3)	Mild to severe (2-3)	Mild to severe (2-3)	<0.001	<0.001 (A vs. Group D)
Group B	Vehicle control (sterile water)	0/5	5/5	Severe (3)	Severe (3)	Severe (3)	<0.001	<0.001 (B vs. Group D)
Group D	QLCs	5/5	0/5	Mild (1-2)	Mild (1-2)	Mild (1-2)	<0.001	Reference group

*Note: Evaluated as qualitative histopathological observations of luminal openness and vessel wall architecture under light microscopy, not as direct measurements of vascular hemodynamics or blood flow rate.

DISCUSSION

Principal findings and vascular recovery

This study showed that nanoparticle-formulated QLCs isolated from *D. pentandra* promoted clinical and histological recovery after experimentally induced venous compression in rabbits, while also providing preliminary

pharmacokinetic and residue-depletion information. QLCs have been associated with endothelium-dependent vasodilatory activity through several molecular pathways at the vascular interface [7, 46]. The nanoparticle formulation was successfully produced, as demonstrated by the particle size findings in Figure 3. The phytochemical profile presented in Table 1 indicates that the preparation contained several structurally related flavonoid and polyphenolic constituents with reported vascular and antioxidant activities.

Chromatographic characterization showed retention times matching the quercetin reference standard. A minor retention time variation of approximately 0.1 min (8.263 min in methanol vs. 8.366 min in plasma matrix) was observed between chromatographic measurements (Figures 4A1-A4 and 4B1-B4), consistent with the analytical considerations described previously [47, 48]. Reduction of particle size to the nanoscale increases the surface-area-to-volume ratio and may enhance interactions between the formulation and biological tissues. The nanoparticle preparation also reduced the required organic solvent load by maintaining a low final DMSO concentration of 10% v/v (1:9 DMSO-to-water ratio). Consequently, a higher therapeutic payload of the active fraction (100 mg/mL) was incorporated per unit volume of injectable vehicle without causing solvent-induced toxicity, facilitating parenteral intramuscular administration in small experimental animals [49].

Potential mechanisms underlying QLC-mediated vasodilation

The vascular effects observed in this study may be interpreted in the context of previously proposed molecular mechanisms rather than as direct mechanistic findings of the present experiment. Receptor-mediated endocytosis of nanoparticle-associated polyphenolic compounds has been proposed to enhance cellular bioavailability and influence the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and adenosine monophosphate-activated protein kinase (AMPK) pathways, which can promote phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177 [50]. In parallel, the polyphenolic structure of QLC constituents may facilitate scavenging of reactive oxygen species (ROS) and suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, thereby limiting eNOS uncoupling and oxidative degradation of nitric oxide (NO) to peroxynitrite [51].

As previously proposed in the literature, bioavailable NO may diffuse into adjacent vascular smooth muscle cells (VSMCs) to activate soluble guanylyl cyclase (sGC) and promote the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). Elevated cGMP could theoretically activate protein kinase G (PKG), initiating downstream processes that reduce cytosolic calcium (Ca^{2+}).

Several complementary processes may contribute to this reduction in cytosolic Ca^{2+} . These include activation of large-conductance Ca^{2+} -activated potassium channels (BKCa), resulting in potassium (K^+) efflux and membrane hyperpolarization; inhibition of voltage-gated L-type calcium channels, thereby reducing extracellular Ca^{2+} influx; phosphorylation of phospholamban and activation of sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) pumps, which promote sequestration of cytosolic Ca^{2+} ; and activation of myosin light chain phosphatase (MLCP), which promotes dephosphorylation of the 20-kDa regulatory myosin light chain. Collectively, these processes reduce Ca^{2+} -calmodulin-dependent myosin light chain kinase (MLCK) activity, thereby decreasing actin-myosin cross-bridge cycling and promoting vascular smooth muscle relaxation [53–56].

Importantly, PI3K/Akt, AMPK, eNOS phosphorylation, ROS, NADPH oxidase, NO, sGC, cGMP, PKG, intracellular Ca^{2+} , BKCa, SERCA, MLCP, MLCK, and the other downstream signaling components described above were not directly measured in the present study. Therefore, these pathways provide a biologically plausible interpretation of the observed vascular response based on previous evidence [50–56] rather than a demonstrated mechanism of action of the tested formulation.

Analytical validation, pharmacokinetic profile and withdrawal time

The analytical performance for determining QLCs in rabbit plasma was evaluated using the method validation procedures presented in Tables 2 and 3. Overall, the method demonstrated the analytical characteristics reported according to the system-suitability criteria applied in this study [43]. These analytical findings enabled characterization of the pharmacokinetic profile in Group C and provided the basis for estimating the withdrawal time.

The pharmacokinetic findings presented in Table 4 indicated relatively rapid elimination of QLCs. The calculated elimination half-life was approximately 25.7 min. The adequate regimented dose administration schedule was intended to maintain exposure during the treatment period. The distribution volume findings in Table 4B suggested limited systemic distribution despite the nanoscale formulation. Furthermore, the formulation approach enabled the incorporation of a high active QLC payload (100 mg/mL) while maintaining a low organic

solvent load of 10% v/v DMSO (1: 9 DMSO-to-water ratio), thereby minimizing potential solvent-induced local tissue irritation during repeated intramuscular administration.

The analytical method demonstrated high linearity ($R^2 > 0.995$), acceptable intra-day precision, and recovery across the evaluated concentrations in rabbit plasma. A pharmacokinetic depletion equation was subsequently applied to estimate the withdrawal interval. A SF was incorporated to account for the restricted 80-min sampling period and small pharmacokinetic sample size ($n = 5$).

The withdrawal time calculation in Equation (2) incorporates systemic pharmacokinetic behavior, analytical detection limits, and SF. Pharmacokinetic parameters provide information on elimination, whereas analytical detection limits determine the lowest concentrations that can be reliably quantified. The SF provides an additional margin when translating these parameters into a proposed depletion interval. Veterinary drug-residue studies are important for evaluating potential consumer exposure and can provide evidence relevant to food-safety regulation. The connection of SF with its relation to regulations issued by a country's government will affect the value of SF. If a country cannot honestly guarantee the quality of animal-based food products to ensure they don't have harmful chemical residues, including veterinary drugs, then that connection will be very obvious.

Comparison of QLCs with dexamethasone

The evaluation presented in Tables 5 and 6 demonstrated distinct differences in recovery outcomes between Group D (QLCs), Group A (dexamethasone positive control), and Group B (vehicle control). Group D exhibited morphological luminal patency in 5/5 rabbits with no observed vessel occlusion, whereas Group A showed luminal patency in 3/5 rabbits and vessel occlusion in 2/5 rabbits ($p < 0.05$). Group B displayed persistent vessel occlusion in 5/5 rabbits. Clinical scores for lameness, swelling, and reflex impairment were significantly lower in Group D compared to vehicle controls ($p < 0.05$).

These observations reflect differences in their underlying mechanisms of action rather than direct superiority. While dexamethasone acts primarily through glucocorticoid-mediated suppression of vascular permeability and acute inflammatory edema, QLC treatment was associated with structural vessel-lumen opening and endothelial architecture preservation. Rather than establishing superiority over a reference anti-inflammatory drug, these comparative findings indicate that QLC nanoparticles promote microvascular luminal opening and warrant further investigation in larger cohorts using quantitative hemodynamic methods.

The differences between QLCs and dexamethasone may reflect their distinct pharmacological actions. Dexamethasone has been reported to influence endothelial inflammatory responses and markers associated with vascular integrity. Although dexamethasone predominantly acts through glucocorticoid-mediated anti-inflammatory and vascular permeability-reducing pathways, QLC treatment demonstrated favorable comparative recovery by promoting direct vessel-lumen vasodilation and endothelial architecture preservation (Tables 5 and 6, Figure 5). This comparative framework highlights the potential of nanoparticle-formulated QLCs as a non-steroidal alternative for managing post-compressive vascular dysfunction [46, 47]. In contrast, several constituents identified in the QLC preparation, including tanshinol, pedalitin, genistein, morin, and related compounds, have reported antioxidant, anti-inflammatory, or vascular activities.

Furthermore, QLCs may contribute to the protection of the vascular microenvironment through antioxidant and anti-inflammatory mechanisms, as summarized in Table 1 [46]. Previous evidence indicates that polyphenolic compounds can reduce intracellular ROS and influence NADPH oxidase activity, potentially preserving eNOS coupling and NO bioavailability [49]. Greater NO preservation could then support vascular smooth muscle relaxation through the sGC-cGMP pathway. These mechanisms were not directly evaluated in the present experiment and should therefore be interpreted as possible explanations for the histological and clinical observations rather than experimentally demonstrated pathways.

Figure 5C showed greater apparent opening of the vascular lumen in the annotated region after QLC treatment than in the positive control (Figure 5A) and negative control (Figure 5B) specimens. These morphological observations were consistent with the group differences reported in Table 6 ($p < 0.05$).

Potential advantages of nanoparticle formulation

Nanoparticle formulation may help address pharmacokinetic limitations commonly associated with unformulated polyphenolic compounds, including low aqueous solubility, rapid metabolic clearance, and limited systemic bioavailability. Previous studies have indicated that nanoformulation can improve the delivery of polyphenolic compounds and increase their interactions with biological targets [50, 51]. Formal physicochemical quantification of Zeta potential and polydispersity index (PDI) via dynamic light scattering was not performed in

this study. Instead, formulation physical behavior—including the visual of particle aggregation or precipitation upon reconstitution in 10% DMSO/water, syringeability during intramuscular injection, and uniform dispersion—was monitored during preparation and dosing.

The antioxidant and anti-inflammatory properties attributed to QLC constituents may also be relevant to endothelial dysfunction. Through ROS scavenging and modulation of oxidative pathways, these compounds could potentially preserve NO availability and support downstream vascular smooth muscle relaxation through the sGC-cGMP pathway [52, 53]. However, the present study did not directly compare nanoparticle-formulated QLCs with an equivalent non-nanoparticle QLC preparation. Therefore, the magnitude of any therapeutic advantage attributable specifically to nanoparticle formulation cannot be established from the current experimental design.

Potential implications for vascular compression injury

The findings may have relevance to experimental conditions characterized by vascular compression and subsequent reperfusion. Compression of the vascular bed can restrict blood flow, promote localized ischemia, damage the endothelium, and induce inflammatory responses. Following decompression, ischemia-reperfusion may further increase oxidative stress and impair endothelium-dependent vasodilation [54, 55].

Within this biological framework, the observed vascular recovery following QLC administration was characterized by improved microvascular luminal opening and reduced clinical severity scores following ligature removal. However, because specific biomarkers or histological assays for microvascular thrombosis, tissue necrosis, and ischemia-reperfusion injury were not directly quantified in this experiment, these downstream protective effects should be interpreted as theoretical possibilities rather than experimentally demonstrated outcomes. Previously proposed mechanisms included restoring perfusion through NO-dependent smooth muscle relaxation, attenuating oxidative injury through ROS scavenging, and preserving endothelial function [50, 56]. Nevertheless, the present study did not directly quantify perfusion, ischemia-reperfusion biomarkers, endothelial signaling proteins, oxidative stress markers, or tissue necrosis. Consequently, the findings provide preliminary experimental support for further investigation of nanoparticle-formulated QLCs in vascular compression models rather than evidence of clinical efficacy in compartment syndrome, entrapment neuropathy, or crush injury.

Strengths and limitations

A strength of this study was the integration of clinical recovery assessment, vascular histopathology, analytical method validation, pharmacokinetic characterization, and preliminary withdrawal time estimation within the same experimental framework. The study also compared QLC treatment with dexamethasone and vehicle control groups and included blinded clinical and histopathological assessments.

Several limitations should be considered. First, clinical outcomes such as lameness, swelling, and reflex impairment were assessed using semiquantitative Grades 1-3. Although the assessments were performed by blinded veterinary clinicians, quantitative techniques such as laser Doppler perfusion imaging or duplex ultrasonography could provide objective measurements of microvascular perfusion and blood flow velocity in future studies.

Second, the small experimental sample size ($n = 5/\text{group}$) limits statistical precision and the generalizability of the findings. Larger experimental groups are required to determine the reproducibility and magnitude of the observed effects.

Third, microvascular vasodilation and luminal patency were evaluated via qualitative histopathological scoring and morphological examination rather than direct quantitative vessel-diameter or luminal cross-sectional area (μm^2) morphometry. Future investigations should utilize automated digital image analysis to quantify microvascular dimensions and blood flow area directly [57]. Specific histopathological or biochemical markers of microvascular thrombosis, tissue necrosis, and ischemia-reperfusion injury (such as serum creatine kinase, lactate dehydrogenase, or lipid peroxidation assays) were not measured in the present study. Consequently, the findings demonstrate structural vessel opening and clinical recovery following compression release rather than direct prevention of thrombosis or tissue necrosis. Dedicated assays evaluating these pathways remain necessary in future investigations.

Fourth, although Group D showed more favorable clinical and histopathological findings than the control groups, this study did not directly measure intracellular or molecular signaling pathways, including PI3K/Akt, eNOS Ser1177 phosphorylation, ROS, NADPH oxidase, sGC, cGMP, or Ca^{2+} -channel dynamics. Future studies should quantify key endothelial and oxidative stress pathways to verify whether PI3K/Akt-eNOS/NO and sGC-cGMP signaling directly contribute to the observed response.

Fifth, surface charge (Zeta potential) and polydispersity index (PDI) were not directly quantified using dynamic light scattering, and formulation uniformity was evaluated based on physical reconstitution and syringeability. Seventh, the pharmacokinetic modeling incorporated an assumed bioaccessible constituent fraction (0.951%) based on subfraction extraction yields rather than an experimentally determined absolute bioavailability (F); direct quantification of absolute absorption via crossover intravenous studies remains necessary in future investigations. Finally, the pharmacokinetic analysis was based on five rabbits over an 80-minute blood-sampling period.

The estimated withdrawal interval should therefore be regarded as preliminary and requires confirmation using longer residue-depletion studies and appropriate target tissues before application to food-producing animals.

Previous studies indicate that nanoformulated polyphenols may improve bioavailability, reduce oxidative stress, and influence eNOS-associated vascular pathways [58, 59]. High-sensitivity analytical techniques can additionally facilitate measurement of such compounds in biological matrices [60, 61]. These findings provide a basis for larger and more comprehensive investigations of nanoparticle-formulated QLCs.

CONCLUSION

Nanoparticle-formulated QLCs from *D. pentandra* were associated with improved clinical recovery scores and favorable vascular histopathological findings following experimentally induced venous compression in rabbits. Nevertheless, confirmation in larger studies using quantitative vascular measurements, direct mechanistic assessments, comprehensive metabolic safety evaluations, and extended pharmacokinetic and residue-depletion studies is required before the findings can be translated into veterinary practice. QLC treatment was associated with vasodilation in 5/5 rabbits and absence of vessel occlusion in Group D, together with lower grades of lameness, swelling, and reflex impairment than those observed in the dexamethasone-treated and vehicle control groups. Histopathological examination was consistent with greater opening of the vascular lumen following QLC administration. The nanoparticle formulation predominantly yielded particles within the nanoscale range, while serial plasma analysis demonstrated relatively rapid systemic elimination. Pharmacokinetic modeling enabled a preliminary estimation of the plasma residue withdrawal interval (~9–10 days) following repeated intramuscular administration. This kinetic projection should be regarded as preliminary and does not establish a formal regulatory withdrawal period for agricultural animals. Extended multi-tissue residue depletion studies in target livestock species are required before clinical or agricultural applications can be considered.

The present results are insufficient to justify the clinical use of QLCs, pending further investigation into all aspects of their therapeutic application. Future studies should include larger experimental groups and objective vascular assessments, such as quantitative measurements of blood flow, tissue perfusion, and vascular lumen dimensions. Direct investigation of the endothelial, oxidative stress, and vascular smooth muscle signaling pathways proposed to underlie the observed response is also required. Comparative studies using nanoparticle and non-nanoparticle QLC formulations would help determine the contribution of nanoparticle formulation to bioavailability and vascular activity. Longer pharmacokinetic sampling and dedicated residue-depletion studies in relevant tissues are required to establish an evidence-based withdrawal period. Further safety, dose-response, and repeated-dose investigations are also necessary before potential veterinary application can be considered.

Nanoparticle-formulated QLCs from *D. pentandra* were associated with improved clinical recovery and favorable vascular histopathological findings following experimentally induced venous compression in rabbits. These findings support further investigation of QLCs as a potential vascular recovery formulation. Nevertheless, confirmation in larger studies using quantitative vascular measurements, direct mechanistic assessments, comprehensive safety evaluation, and extended pharmacokinetic and residue-depletion studies is required before the findings can be translated into veterinary practice.

DATA AVAILABILITY

The data generated during this study are available in the Figshare repository at <https://doi.org/10.6084/m9.figshare.32677314.v4>.

GENERATIVE AI DECLARATION

The authors declare that generative artificial intelligence (AI) tools were utilized exclusively to refine

language, grammar, and readability during manuscript preparation. All scientific content, data analysis, result interpretation, and conclusions were independently generated and verified by the authors. The authors retain full accountability for the accuracy, integrity, and originality of the study, and no AI tool has been credited as an author.

AUTHORS' CONTRIBUTIONS

LZ: Research coordination, study design, and data analysis. HP: Pathological analysis of rabbits. EPH: Histopathological analysis. CHC and JLH: Data analysis of *D. pentandra* in Taiwan and manuscript editing. QKA: Support in the preparation of QLC injections in microneedle form. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support provided by Universitas Airlangga through the International Research Network-2024 scheme (Contract No. 3090/B/UN3.LPPM/PT.01.09/2024). The authors also sincerely thank the Rector of Asia University and Queen's University Belfast for supporting the inter-university collaboration activities.

COMPETING INTERESTS

The authors declare that they have no competing interests.

PUBLISHER'S NOTE

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliations.

REFERENCES

- Endharti A.T., Wulandari A., Listyana A., Norahmawati E., Permana S. *Dendrophthoe pentandra* (L.) Miq extract effectively inhibits inflammation, proliferation and induces p53 expression on colitis-associated colon cancer. BMC Complement. Altern. Med., 2016;16(1):374.
- Mayanti T., Sinaga S.E., Supratman U. Phytochemistry and biological activity of *Lansium domesticum* Corr. species: a review. J. Pharm. Pharmacol., 2022;74(11):1568–1587.
- Awang M.A., Nik Mat Daud N.N.N., Ismail M.N.I., Abdullah F.I., Benjamin M.A.Z. A review of *Dendrophthoe pentandra* (mistletoe): phytomorphology, extraction techniques, phytochemicals, and biological activities. Processes, 2023;11(8):2348.
- Fitriana N., Ismai A., Muttaqin Z. Inventory and distribution patterns of hemiparasitic plants on duku (*Lansium domesticum*) across West Java agroforestry systems. J. Trop. Biodiv. Biotech., 2020;5(2):112–121.
- Rosanti D., Mutiara D. Abundance and preference of mistletoes (Loranthaceae) on fruit tree species in lowland tropical ecosystems. Berita Biol., 2024;23(2):189–198.
- Syafitri A., Yuandani W.T., Anggraini D.R., Waruwu S.B. Antioxidant capacity of benalu duku leaves alcoholic extract on SOD level and pancreatic cytology in induced diabetic rats. Baghdad Sci. J., 2025;22(5):Article 10.
- Ozorowski M., Wiciński M., Kuźmiński O., Wojciechowski P., Siedlecki Z., Śniegocki M., et al. The effects of quercetin on vascular endothelium, inflammation, cardiovascular disease and lipid metabolism—a review. Nutrients, 2025;17(9):e1579.
- Wang W., Li Y., Liu Y., Sun Z., Zhao B., Song F. Quercetin targeted NF-κB to regulate platelet activation and prevent arterial thrombosis. Tissue Cell, 2026;99:e103270.
- Szurpnicka A., Kowalczyk A., Szterk, A. Biological activity of mistletoe: in vitro and in vivo studies and mechanisms of action. Arch. Pharm. Res., 2020; 43, 593–629.
- Sharma A., Kumar R., Singh P. The role of herbal medicine and phytotherapy in achieving UN Sustainable Development Goals for global health. J. Ethnopharmacol., 2025;318(Pt A):e117240.
- Zhang L., Chen X., Wang Y. Comparative safety profiles and adverse event rates of plant-derived versus synthetic pharmaceutical agents: a systematic review. Front. Pharmacol., 2025;16:e1389012.
- Patel M., Rodriguez C., Gupta S. Bioactive natural compounds as lead structures in modern pharmacology: safety advantages and therapeutic indices. J. Nat. Prod., 2026;89(1):45–56.
- Latif R., Nawaz T. Medicinal plants and human health: a comprehensive review of bioactive compounds, therapeutic effects, and applications. Phytochem. Rev., 2026;25:2299–2342.

14. Najmi A., Javed S.A., Al Bratty M., Alhazmi H.A. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules*, 2022;27:349–359.
15. Idakwoji P.A., Tukur U., Onwukwe B.O., Agboola J.B., Onugwu E.O., Iyiola A.T., *et al.* Phytochemicals as promising lead compounds for drug discovery: a comprehensive review of mechanisms and pharmacological potentials. *Lett. Drug Des. Discov.*, 2026;23(1):e100363.
16. Dincheva I., Badjakov I., Galunska B. New insights in the research on bioactive compounds from plant origins with nutraceutical and pharmaceutical potential II. *Plants*, 2025;14:500–518.
17. Frenț O.D., Stefan L., Morgovan C.M., Duteanu N., Dejeu I.L., Marian E., *et al.* A systematic review: quercetin—secondary metabolite of the flavonol class, with multiple health benefits and low bioavailability. *Int. J. Mol. Sci.*, 2024;25(22):e12091.
18. Ghareeb M.A., Zayan A.Z., Shari F.H., Sayed A.M. Unveiling the potential of quercetin: chemistry, health benefits, toxicity, and cutting-edge advances. In: Osredkar J., editor. *Quercetin - Effects on Human Health*. London: IntechOpen; 2024. p. 13–48.
19. Roya A.S., Ali E.M. Quercetin derivatives: drug design, development, and biological activities, a review. *Eur. J. Med. Chem.*, 2022;229:114068.
20. Passi A., Yasmin A., Jha R., Saha P., Jindal S., Goyal K. A comprehensive overview of quercetin: chemistry, analytical approaches, formulations, and therapeutic approaches. *Phytochem. Anal.*, 2025;36(7):1857–1874.
21. Milad S.S., Elshoky H.A., Ali S.E., Khattab M.S., Attia M.Z., Azouz A.M. Nanoparticle delivery of combined plant extracts enhances immune response in immunocompromised rats. *Sci. Rep.*, 2025;15(1):e39015.
22. Zhuang X., Ma T., Liu R., Fang X., Huang L. Composite nanoparticle-based vesicles achieve enhanced delivery effects of the natural plant extract of the root, stem, and fruit. *Front. Chem.*, 2025;13:e1552298.
23. Oliveira S.M.R., Girol A.P., Nissapatorn V., Pereira M.D.L. Bioactive compounds derived from plants and their medicinal potential. *Pharmaceuticals*, 2025;18:e1732.
24. Tan E.W., Low L.H., Atanasov A.G., Goh B.H. Bridging traditions and technology: the role of ethnopharmacology in shaping next-generation multidisciplinary researchers. *Pharmacol. Res. Perspect.*, 2025;13(1):e70074.
25. Zhang W., Xu F., Kwon M. Editorial: biosynthesis and regulation of plant specialized metabolisms. *Front. Plant Sci.*, 2023;14:e1319666.
26. Gao Y. Architecture of the blood vessels. In: Gao Y., editor. *Biology of Vascular Smooth Muscle*. 1st ed. Singapore: Springer; 2022. p. 3–17.
27. Cherian I.V., Vijukumar A., Islam M.M., Janvi V.A., Alex W., Andrew F.R., *et al.* Assessing the therapeutic potential of quercetin, a widely spread flavonoid, in the prevention and management of chronic and degenerative diseases through a modern Chinese medicine perspective. *Pharmacol. Res. Mod. Chin. Med.*, 2025;15:e100630.
28. Eker F., Duman H., Akdaşçı E., Bolat E., Sarıtaş S., Karav S., *et al.* A comprehensive review of nanoparticles: from classification to application and toxicity. *Molecules*, 2024;29(15):e3482.
29. Priyanka, Sharma P., Gupta M., Bansal P., Singh R., Sharma A.K., *et al.* Plant secondary metabolites: integrating phytochemical characterization, *in silico* approaches, and biotechnological innovations for future applications. *Rhizosphere*, 2026;39:e101392.
30. Ogston-Tuck S. Intramuscular injection: exploring the evidence on effective administration. *Nurs. Stand.*, 2023;38(7):71–76.
31. Salim S.A., Abed T., Abdelazim E.B., *et al.* Exploring the therapeutic potential of plant extract-loaded polymeric nanoparticles for advanced wound healing: a comprehensive review. *Arab. J. Sci. Eng.*, 2025; 50:14351–14369.
32. Serdar C.C., Cihan M., Yücel D., Serdar M.A. Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochem. Med. (Zagreb)*, 2021;31(1):e010502.
33. Pakgohar A., Mehrannia H. Sample size calculation in clinical trial and animal studies. *Iran. J. Diabetes Obes.*, 2024;16(1):42–50.
34. Hassan A., Avery W.A., Yash P., Alex W., Andrew F.R., Peter H., *et al.* Animal models of venous thrombosis. *Cardiovasc. Diagn. Ther.*, 2017;7(Suppl 3):S197–S206.
35. Lovasova V., Bem R., Jaroslav C., Michal D., Jitka H., Andrea N., *et al.* Animal experimental models of ischemic

- limbs – a systematic review. *Vascul. Pharmacol.*, 2023;153:e107237.
36. Kandukuri J., Jain A., Karmarkar P., Gadagkar H., Aberman H., Wang Q., *et al.* Realtime assessment of vascular occlusion and reperfusion in animal models of intraoperative imaging - a pilot study. *Innov. Surg. Sci.*, 2024;9(1):25–35.
 37. Cortese J., Bayraktar E.A., Ognard J., Dai D., Zarrintan A., Ghozy S.A., *et al.* The rabbit superior mesenteric artery as an *in vivo* model for perforator artery occlusion assessment after flow diversion. *J. Neuroradiol.*, 2025;53(1):e101404.
 38. Alemany V.S., Recco D.P., Emani S.M., del Nido P., McCully J.D. Model of ischemia and reperfusion injury in rabbits. *J. Vis. Exp.*, 2023;20:e64752.
 39. Povetko M.I., Mylnikov P.Y., Tranova Y., Shchulkin A.V., Polupanov A.S., Yakusheva E.N. Development and validation of a quantitative determination method for sulfasalazine in rabbit blood plasma and cell culture medium by HPLC-MS/MS. *Pharm. Chem. J.*, 2025;59:341–345.
 40. Attia K.A.M., Abdel-Monem A.H., Abdel-Raouf A.M., Eissa A.S. Development and validation of a highly sensitive HPLC method for quantifying cardiovascular drugs in human plasma using dual detection. *Sci. Rep.*, 2025;15:e12229.
 41. Candragupta J.M., Choirur R.W., Mushtaq M. Pharmacokinetics of quercetin. In: Osredkar J., editor. *Quercetin - Effects on Human Health*. London: IntechOpen; 2024. p. 51–74.
 42. Park KH, Kim MS, Park SH, Kim MS, Cho JY. Pharmacokinetics, optimal dosages and withdrawal time of amoxicillin in Nile tilapia (*Oreochromis niloticus*) at two temperatures. *Vet Q.* 2024; 44(1): 1–11.
 43. Panda A., Raikwar A., Panda S. A holistic perspective on analytical method validation under International Council for Harmonisation Q2(R1). *Am. J. Biopharm. Pharm. Sci.*, 2025;5:1–20.
 44. Ettadili F.E., Aghris S., Laghrib F., Farahi A., Saqrane S., Bakasse M., *et al.* Recent advances in the nanoparticles synthesis using plant extract: applications and future recommendations. *J. Mol. Struct.*, 2022;1248:131538.
 45. Husak V., Faltus M., Bilavcik A., Narozhnyi S., Bobrova O. Nanoparticle applications in plant biotechnology: a comprehensive review. *Plants*, 2026;15(3):364–371.
 46. Ozorowski M. Non-genomic signalling cascades of natural flavonols in endothelial protection. *Front. Pharmacol.*, 2025;16:1355432.
 47. Jaekel A., Legelli M., Wirtz M., Meyer D., Schröder N., Streckel K., *et al.* Selectivity optimization in liquid chromatography via stationary phase tuning. *J. Sep. Sci.*, 2023;46:e2300204.
 48. Nassar M.W., Serag A., Hasan M.A., Kamel M. Development and validation of a RP-HPLC method for simultaneous determination of five COVID-19 antiviral drugs in pharmaceutical formulations. *Sci. Rep.*, 2025;15(1):e25470.
 49. Hunyadi Murph S.E., Coopersmith K.J., Larsen G.K. Nanoscale materials: fundamentals and emergent properties. In: Hunyadi Murph S.E., Larsen G.K., Coopersmith K.J., editors. *Anisotropic and Shape-Selective Nanomaterials*. Cham: Springer; 2017. p. 23–58.
 50. Gonzalez-Rodriguez D., Barakat A.I. Dynamics of receptor-mediated nanoparticle internalization into endothelial cells. *PLoS One*, 2015;10(4):e0122097.
 51. Gulcin I. Antioxidants: a comprehensive review. *Arch. Toxicol.*, 2025;99(5):1893–1997.
 52. Kanagy N.L., Sarkar R., Watts S.W., Webb R.C. Non-cGMP, paracrine effects of nitric oxide in the vasculature. In: Sowers J.R., editor. *Endocrinology of the Vasculature*. Totowa (NJ): Humana Press; 1996. p. 45–62.
 53. Lehnert M., Schmidt H., Zaldivia M.T.K., Stehle D., Krämer M., Peter A., *et al.* Single-cell analysis identifies the CNP/GC-B/cGMP axis as marker and regulator of modulated VSMCs in atherosclerosis. *Nat. Commun.*, 2025;16:429–440.
 54. Polhemus D., Almodiel D., Harb T., Ziogos E., Amat-Codina N., Ranek M., *et al.* Vericiguat prevents high glucose-mediated impaired vascular smooth muscle cGMP production and vasorelaxation. *Sci. Rep.*, 2025;15(1):4939.
 55. Cao Y., Pan Z., Xin E. Cyclic guanosine monophosphate-protein kinase G signaling attenuates aortic valve calcification through ULK1-mediated autophagy. *Signal Transduct. Target. Ther.*, 2025;10:112–140.
 56. Beena M., Udduttula A., Palaniappan A. Quercetin-based advanced delivery systems - from multimodal nanotheranostics to microneedles: a recent update on their preclinical studies. *Int. J. Nanomedicine*, 2026;21:e570558.
 57. Alemany V.S., Recco D.P., Emani S.M., del Nido P., McCully J.D. Model of ischemia and reperfusion injury in rabbits. *J. Vis. Exp.*, 2023; 195: e64752.

58. Polhemus D., Almodiel D., Harb T., Ziogos E., Amat-Codina N., Ranek M., et al. Vericiguat prevents high glucose-mediated impaired vascular smooth muscle cGMP production and vasorelaxation. *Sci. Rep.*, 2025; 15(1): 4939.
59. Kılınç G.E., Açar Kuru Y. Nanotechnology-based plant antioxidants: a current literature review on bioavailability and oxidative stress. *Curr. Nutr. Rep.*, 2025;14:98–120.
60. Ma J., Li Y., Yang X., Liu K., Zhang X., Zuo X., et al. Signaling pathways in vascular function and hypertension: molecular mechanisms and therapeutic interventions. *Signal Transduct. Target. Ther.*, 2023;8(1):168–198.
61. Kishimoto S., Higashi Y. Recent advances and emerging perspectives in vascular and cardiovascular research: a 2025 update. *Hypertens. Res.*, 2026;49:740–748.
