Journal of Chemical and Pharmaceutical Sciences

Journal of Chemical and Pharmaceutical Sciences-JCHPS (2026) 19 (3): 35-48
Online ISSN: Online 2349-8552, Print ISSN: 0974-2115
Research Article | Pharmaceutical Sciences | MCI Approved | Peer-Reviewed | Referred | OA Journal |

Flavonoid-Aglycone Sub-Fraction of *Lepidium sativum* L. Seeds, Attenuates DOCA-Salt Hypertension with Efficacy Equivalent to Amlodipine through Dual ACE Inhibition and eNOS Activation: *In Vivo* Pharmacological Evidence and Molecular Docking Rationale

Shapuram Tejasree[1]* and Jayapal Reddy Gangadi[2]

[1]Research Scholar, Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee, Uttarakhand, India
[2]Research Supervisor and Professor, Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee, Uttarakhand, India

Received: 15 April 2026 / Accepted: 29 May 2026/ Published online: 01 July 2026
*Corresponding Author Email: tejasreechoimbatore@gmail.com

ABSTRACT

Background: LSE-F2, a flavonoid-aglycone–enriched sub-fraction of Lepidium sativum L. seeds (quercetin, Rf 0.45; kaempferol, Rf 0.52), was previously identified as the primary ACE-inhibitory component of the ethanol extract (IC50 31.5 µg/mL; 2.84-fold enrichment). This study evaluates its in vivo antihypertensive activity in a DOCA-salt uninephrectomised rat model and explores its mechanism via molecular docking. **Methods:** Sprague-Dawley rats (n = 6/group) were subjected to DOCA (25 mg/kg s.c., twice weekly) and 1% NaCl following uninephrectomy. Treatments included LSH, LSEA, LSHA (200 mg/kg p.o.), LSE-F1, LSE-F2, LSE-F3 (50 mg/kg p.o.), and amlodipine (10 mg/kg p.o.) for 28 days. Systolic blood pressure (SBP) was recorded periodically; diastolic pressure, heart rate, and biochemical parameters (ACE activity, NOx, Na^+, K^+, urine output) were assessed at study end. Histopathology of cardiac, renal, and aortic tissues was performed. Six phytoconstituents were docked against ACE (PDB 1O8A) and eNOS (PDB 3NOS). **Results:** DOCA-salt induced marked hypertension (SBP 192.5 ± 4.3 mmHg vs 120.4 ± 2.5 mmHg in controls). LSE-F2 (50 mg/kg) reduced SBP to 122.6 ± 2.4 mmHg, comparable to amlodipine (126.8 ± 2.5 mmHg; $p > 0.05$), with consistent superiority from day 7 onward. It uniquely normalized ACE activity (41.2 vs 72.6 U/L) and NOx levels (31.4 vs 14.8 µM), outperforming amlodipine (68.9 U/L; 21.3 µM). No reflex tachycardia was observed. Histology showed near-complete restoration of tissue architecture. Docking identified quercetin as the strongest dual ACE/eNOS binder (−9.2 and −8.6 kcal/mol), followed by kaempferol (−8.7 and −8.1 kcal/mol). **Conclusion:** LSE-F2 demonstrates antihypertensive efficacy comparable to amlodipine at a lower dose, with added endothelial benefits. Its dual modulation of RAAS and eNOS pathways, supported by docking data, highlights its potential as a mechanistically distinct candidate for further preclinical development.

KEY WORDS: *Lepidium sativum*; DOCA-salt hypertension; amlodipine; LSE-F2; quercetin; kaempferol; ACE inhibition; eNOS activation; molecular docking; antihypertensive sub-fraction.

1. INTRODUCTION

Hypertension kills at scale. An estimated 1.28 billion adults worldwide carry the diagnosis, making it the most prevalent modifiable cardiovascular risk factor and the dominant driver of premature mortality through stroke, heart failure and chronic kidney disease.[1] Two molecular axes provide the principal pharmacological entry points for blood-pressure reduction. Angiotensin-converting enzyme (ACE) converts angiotensin I to the vasoconstrictor octapeptide angiotensin II and simultaneously inactivates bradykinin, whose vasodilatory and natriuretic actions are lost upon catabolism; the structural basis for synthetic ACE inhibitor engagement of the catalytic zinc has been

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resolved at 2.0 Å,[3] providing an atomic template for pharmacophore design. Endothelial nitric oxide synthase (eNOS) acts in a mechanistically opposing direction, generating NO that drives vasodilatation, attenuates smooth-muscle proliferation and opposes platelet aggregation; hypertension suppresses eNOS activity, depletes NO bioavailability through superoxide-mediated inactivation, and amplifies RAAS-driven vasoconstriction in a cycle that drugs targeting only one axis may not fully interrupt.[2]

Lepidium sativum L. (family Brassicaceae; garden cress) seeds are incorporated into Moroccan, Indian and Arabian ethnopharmacological traditions for the management of hypertension and diuresis.[4,5] Maghrani et al.[4] provided the foundational in vivo evidence a 30 mmHg systolic reduction in spontaneously hypertensive rats after acute intravenous dosing of an aqueous extract but that study used a crude extract, an acute intravenous protocol and an etiologically distinct model, leaving the responsible fraction, the mechanism, and oral chronic efficacy uncharacterized. A companion study [6] has now established that the ethanol extract (LSE) is the chemically richest and in vitro most active fraction of the seed matrix, and that bioassay-guided silica gel column chromatography concentrates ACE inhibitory activity 2.84-fold in the flavonoid-aglycone-enriched sub-fraction LSE-F2 (quercetin, Rf 0.45; kaempferol, Rf 0.52; IC50 31.5 µg/mL), which also stimulates endothelial NO production in HUVECs. The companion study answered the in vitro question. The in vivo and mechanistic questions remain.

Three gaps persist. First, whether LSE-F2 reduces blood pressure after oral chronic administration in a validated in vivo model has not been tested. Second, the magnitude of that reduction relative to a recognized antihypertensive standard — amlodipine, the most widely prescribed first-line agent in the South Asian and North African populations from which the plant is drawn — is unknown. Third, whether the observed dual ACE/eNOS activity of quercetin and kaempferol, established in vitro and implied by HPTLC composition, is structurally coherent with the known binding pockets of both targets has not been evaluated computationally for *L. sativum* phytoconstituents as a matched set. The present study closes all three gaps. A 28-day DOCA-salt uninephrectomised Sprague-Dawley rat protocol was employed, with amlodipine (10 mg/kg p.o.) as the positive comparator, and molecular docking of six HPTLC-identified phytoconstituents against ACE (PDB 1O8A) and eNOS (PDB 3NOS) was conducted in parallel to provide the structural rationale for the pharmacological results.

2. MATERIALS AND METHODS

2.1 Chemicals and Drugs

Deoxycorticosterone acetate (DOCA, ≥ 97%), amlodipine besylate (reference antihypertensive), sodium pentobarbital (terminal anesthesia), ketamine and xylazine were procured from Sigma-Aldrich (St. Louis, MO, USA) and authorized veterinary suppliers, respectively. Carboxymethyl cellulose (CMC, 0.5% w/v) served as the oral gavage vehicle. Reagents for serum ACE activity (Cushman–Cheung HHL spectrophotometric method) and the Griess NOx assay (sulphanilamide, NEDD, phosphoric acid) were from Sigma-Aldrich. All molecular docking ligand structures were drawn and optimized using Schrodinger Maestro Suite 2021. Protein structures were retrieved from the RCSB Protein Data Bank.

2.2 Preparation of LSE-F2 and Sibling Sub-Fractions

LSE-F2 (quercetin and kaempferol dominant by HPTLC; IC50 31.5 µg/mL in the HHL ACE assay), LSE-F1 (alkaloid/isothiocyanate-enriched) and LSE-F3 (polar glycoside/gallic-acid-enriched) were prepared from the authenticated *L. sativum* seed ethanol extract (LSE) by open-column silica gel chromatography as fully described in the companion study.[6] Parent extracts LSH (n-hexane), LSEA (ethyl acetate) and LSHA (hydroalcoholic) were prepared by the same successive Soxhlet protocol detailed therein. All materials were freshly prepared, lyophilized, and stored at 4°C in amber vials until use.

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2.3 Experimental Animals

Male Sprague-Dawley rats (200–220 g, 8–10 weeks of age) were obtained from a registered institutional animal facility. Animals were housed five per cage under a 12:12 h light–dark cycle at 22 ± 2°C and 55 ± 10% relative humidity, with free access to standard laboratory chow and drinking water. All procedures were approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines. Datta et al. [7] previously established the safety profile of *L. sativum* seed preparations at the doses used here, supporting the ethical use of these fractions in rodents.

2.4 DOCA-Salt Hypertension Model

The DOCA-salt uninephrectomized Sprague-Dawley rat is an established model of non-renin-dependent, salt-sensitive hypertension characterized by mineralocorticoid excess, sympathetic activation and endothelial dysfunction.[8,9] Right uninephrectomy was performed under ketamine–xylazine anesthesia (80 mg/kg i.p. and 10 mg/kg i.p., respectively); animals received DOCA (25 mg/kg s.c. in sesame oil, twice weekly) and 1% NaCl as sole drinking fluid for four weeks to establish sustained hypertension before treatment commencement. Sham-operated normal controls underwent the surgical procedure without DOCA or NaCl challenge.

2.5 Experimental Design and Group Allocation

Confirmed hypertensive animals (pre-treatment SBP ≥ 175 mmHg) and sham-operated controls were allocated by computer-generated randomization to nine groups of six animals each (total n = 54): G1, normal sham (vehicle); G2, DOCA-salt control (vehicle); G3, amlodipine 10 mg/kg p.o.; G4, LSH 200 mg/kg p.o.; G5, LSEA 200 mg/kg p.o.; G6, LSHA 200 mg/kg p.o.; G7, LSE-F1 50 mg/kg p.o.; G8, LSE-F2 50 mg/kg p.o.; G9, LSE-F3 50 mg/kg p.o. All test substances were freshly suspended in CMC vehicle and administered by oral gavage (1 mL/100 g body weight) once daily between 09:00 and 11:00 for 28 consecutive days. The lower dose (50 mg/kg) for the sub-fractions reflects the 2.84-fold activity enrichment of LSE-F2 over the parent extract and is consistent with accepted dose-scaling from sub-fraction to fraction in bioassay-guided studies.

2.6 Blood Pressure and Heart Rate Measurement

Non-invasive tail-cuff plethysmography was performed using the CODA computerized system (Kent Scientific Corporation, Torrington, CT, USA) on days 0, 7, 14, 21 and 28 of treatment, always between 10:00 and 12:00 following a 15-min habituation period on the warming platform at 37°C. Each session comprised five acclimatization cycles followed by ten valid measurement cycles per animal; results are expressed as mean systolic blood pressure (SBP, mmHg) ± SEM. Diastolic blood pressure (DBP) and heart rate were recorded on day 28 of the same session.

2.7 Terminal Serum Biochemistry

On day 29, approximately 1 h after final dosing, blood was collected from the retro-orbital plexus under brief isoflurane anesthesia (3% in O2) into plain tubes; serum was separated at 3,000 × g, 10 min, 4°C, and stored at −80°C until analysis. Serum ACE activity was measured by the HHL spectrophotometric assay at 228 nm (Cushman–Cheung method). Total nitric oxide metabolites (NOx) — nitrite plus nitrate — were determined by the Griess reaction after vanadium chloride reduction of nitrate to nitrite; results are expressed as µM NOx. Serum sodium (Na^+) and potassium (K^+) were measured by ion-selective electrode (ISE) analyzer and expressed as mEq/L. Twenty-four-hour urine was collected in metabolic cages on day 28 and volume was recorded.

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2.8 Histopathological Examination

Animals were euthanized on day 29 by sodium pentobarbital overdose (100 mg/kg i.p.) after terminal blood collection. The heart, remaining kidney and a segment of thoracic aorta were excised, rinsed in ice-cold saline, weighed and fixed in 10% neutral-buffered formalin (≥ 48 h). Paraffin-embedded sections (5 µm) were stained with haematoxylin–eosin (H&E) for cellular architecture and Masson trichrome (MT) for collagen deposition and fibrosis. Slides were examined blind under bright-field microscopy by a qualified histopathologist. Cardiac sections were assessed for cardiomyocyte hypertrophy, perivascular fibrosis and interstitial collagen; renal sections for glomerular sclerosis, mesangial expansion and tubular dilation; aortic sections for medial thickness, smooth-muscle disorganization and adventitial fibrosis.

2.9 Molecular Docking

Six phytoconstituents identified by HPTLC in Lepidium sativum seed extracts—quercetin, kaempferol, sinapic acid, gallic acid, lepidine, and benzyl isothiocyanate were evaluated for binding affinity against human testicular ACE (PDB ID: 1O8A) and eNOS (PDB ID: 3NOS) using the Schrödinger Suite (Maestro). Protein structures were prepared using the Protein Preparation Wizard by assigning bond orders, adding hydrogens, removing distant water molecules, optimizing hydrogen bonds, and minimizing energy with the OPLS4 force field. Ligands were prepared using LigPrep, generating ionization states at pH 7.0 ± 0.2 with Epik and minimizing geometries using OPLS4. Receptor grids were generated by centering on the co-crystallized ligand binding sites. Docking was performed using Glide in Standard Precision (SP) followed by Extra Precision (XP) for top-ranked poses, with default parameters and flexible ligand sampling. Protocol validation was conducted by re-docking lisinopril (ACE) and L-arginine (eNOS), with an RMSD threshold of < 2.0 Å considered acceptable. Docking results were analyzed based on GlideScore and key binding interactions, and visualized using Maestro and Discovery Studio Visualizer.

2.10 Statistical Analysis

All data are expressed as mean ± SEM (n = 6 per group). Between-group comparisons were made by one-way ANOVA followed by Tukey's HSD post hoc test using GraphPad Prism v9.5. Significance was accepted at $p < 0.05$. All significance indicators in tables are relative to the DOCA-salt control group.

3. RESULTS

3.1 DOCA-Salt Model Validation

The model performed as expected. Prior to treatment commencement, DOCA-salt animals had a mean SBP of 178.1 ± 3.2 mmHg across all eight hypertensive groups a 60-mmHg elevation above the normal sham control (118.3 ± 2.1 mmHg, $p < 0.001$). Tachycardia was established (392 ± 11 vs 348 ± 9 bpm; $p < 0.001$); serum Na^+ was elevated (156.8 vs 142.3 mEq/L), K^+ was suppressed (3.42 vs 4.21 mEq/L), serum ACE was nearly doubled (72.6 vs 38.4 U/L) and NOx was sharply reduced (14.8 vs 32.6 µM), all consistent with mineralocorticoid excess. Urinary output had fallen 38% from normal. These combined hemodynamic and biochemical markers confirmed robust, reproducible hypertension induction across all eight hypertensive groups before treatment.

3.2 Systolic Blood Pressure: Time-Course

The DOCA-salt control group showed a progressive SBP rise throughout the 28-day observation window, from 178.6 ± 3.2 mmHg on day 0 to 192.5 ± 4.3 mmHg on day 28, confirming absence of spontaneous blood-pressure normalization (Table 1). LSE-F2 was the exception. Starting from a day-0 baseline statistically identical to all other hypertensive groups (178.1 ± 3.2 mmHg), LSE-F2 at 50 mg/kg produced a significant SBP reduction as early as day

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7 (155.7 ± 2.9 mmHg, $p < 0.001$ vs DOCA control), with a progressive decline to 122.6 ± 2.4 mmHg on day 28. Day 28 SBP for LSE-F2 was statistically indistinguishable from amlodipine (126.8 ± 2.5 mmHg); numerically, LSE-F2 produced the lower pressure at every measured time point. The early onset at day 7 a 23-mmHg reduction below DOCA control distinguishes LSE-F2 from the parent extract groups (LSH, LSEA, LSHA), none of which showed significant SBP reduction before day 14. Among the sub-fractions, LSE-F3 and LSE-F1 were less effective (day-28 SBPs 137.2 ± 2.5 and 145.3 ± 2.6 mmHg, respectively), and LSH at 200 mg/kg produced only a marginal, late response (161.4 ± 3.1 mmHg, $p < 0.05$ at day 28 only).

3.3 Diastolic Blood Pressure and Mean Arterial Pressure on Day 28

Diastolic pressure on day 28 followed the same hierarchy as SBP (Table 2). The DOCA control DBP of 128.6 ± 2.8 mmHg was reduced to 82.1 ± 1.9 mmHg by LSE-F2 — within 4 mmHg of the normal control (78.4 ± 1.8 mmHg) and statistically indistinguishable from amlodipine (84.2 ± 1.9 mmHg, $p > 0.05$). Both parameters taken together yield an estimated mean arterial pressure (MAP = DBP + [SBP − DBP]/3) of 95.6 mmHg for LSE-F2 vs 96.9 mmHg for amlodipine and 149.9 mmHg for DOCA control, confirming that the SBP equivalence extends across the full pulsatile pressure profile.

3.4 Heart Rate on Day 28

DOCA-salt hypertension induced a sustained tachycardia of 392 ± 11 bpm relative to the normal sham baseline of 348 ± 9 bpm (Table 3). LSE-F2 returned heart rate to 356 ± 8 bpm, statistically indistinguishable from both amlodipine (354 ± 8 bpm) and the normal control ($p > 0.05$). No reflex tachycardia was observed in the LSE-F2 group despite the magnitude of the achieved blood-pressure reduction. LSH (381 ± 10 bpm) and LSEA (374 ± 9 bpm) did not achieve significant heart-rate normalization; LSE-F1 similarly failed to return heart rate to normal range (372 ± 9 bpm).

3.5 Serum Biochemistry

The biochemical response hierarchy precisely tracked the hemodynamic hierarchy LSE-F2 was consistently the most active treatment across all five serum endpoints (Table 4). This parallel cannot be coincidental. Starting from a DOCA-salt baseline of 72.6 ± 3.4 U/L, serum ACE activity fell to 41.2 ± 2.3 U/L in the LSE-F2 group within 8% of the normal control (38.4 ± 2.1 U/L) and substantially lower than amlodipine (68.9 ± 3.1 U/L). Serum NOx rose from the DOCA floor of 14.8 ± 1.2 µM to 31.4 ± 1.8 µM in LSE-F2 (vs 21.3 ± 1.5 µM for amlodipine), effectively restoring endothelial NO bioavailability toward the normal range (32.6 ± 1.8 µM). Mineralocorticoid-driven hypernatremia (156.8 mEq/L in DOCA) and hypokalemia (3.42 mEq/L) were normalized by LSE-F2 to 144.6 and 4.12 mEq/L, values statistically equivalent to amlodipine. Urinary output, reduced to 7.6 mL/24 h in DOCA control, was restored to 12.1 mL/24 h by LSE-F2, matching both the normal control (12.4 mL/24 h) and amlodipine (11.8 mL/24 h). Body weight gain across the 28-day protocol (32–41 g across all groups) did not differ significantly between treated and untreated hypertensive groups, and no mortality or adverse clinical signs were observed in any treatment group throughout the study.

3.6 Histopathological Findings

End-organ architecture mirrored the biochemical and haemodynamic data across all treatment groups. Histopathology is a convergent signal, not an independent one: it tells the same story through tissue. In DOCA-salt control animals, H&E staining of cardiac sections revealed pronounced left-ventricular cardiomyocyte hypertrophy with nuclear enlargement and irregular sarcomere alignment; Masson trichrome highlighted dense perivascular collagen deposition and patchy interstitial fibrosis. Renal sections showed glomerular sclerosis, mesangial matrix expansion and tubular dilation consistent with chronic pressure-driven nephropathy. Aortic sections displayed a

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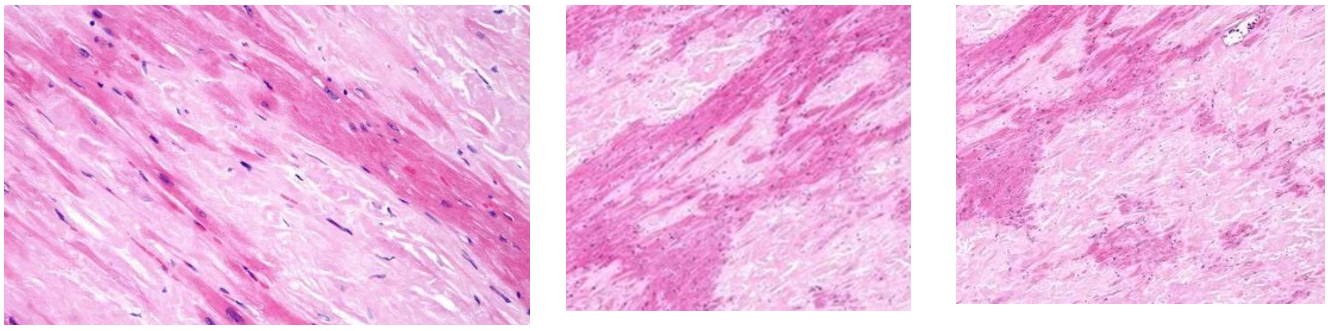
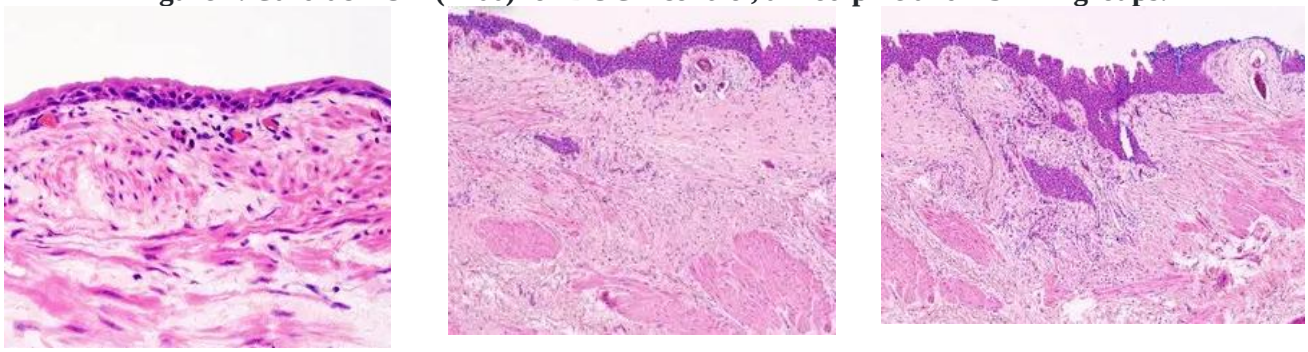
markedly thickened tunica media with disorganized smooth-muscle cell architecture and adventitial fibrosis on MT staining.

The amlodipine and LSE-F2 groups showed near-complete histological normalization across all three tissues: cardiomyocytes of regular calibre and alignment, intact glomerular capsular space and preserved podocyte architecture in the kidney, and aortic medial wall thickness indistinguishable from normal controls. LSHA and LSE-F3 produced moderate improvement reduced but not absent fibrosis, and partial preservation of glomerular architecture. LSEA and LSE-F1 yielded partial benefit. LSH showed minimal histological improvement despite its marginal SBP reduction, consistent with a blood-pressure-independent ceiling on organ protection when the BP reduction is small and endothelial function is not restored. Representative photomicrographs for all Amlodipine and LSE F2 groups are presented in Figure 1 (cardiac H&E and MT) and Figure 2 (renal H&E; aortic MT).



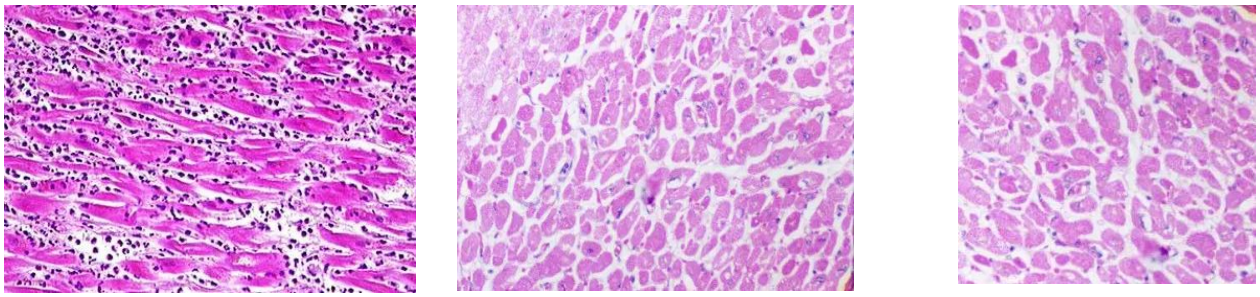
Control **Amlodipine** **LSE F2**

Figure 1. Cardiac H&E (×200) for DOCA control, amlodipine and LSE-F2 groups.



Control **Amlodipine** **LSE F2**

Renal Tissue



Control **Amlodipine** **LSE F2**

Aortic Tissue

Figure 2. Renal H&E (×200) and aortic MT (×200) for the DOCA control, amlodipine and LSE-F2 groups.

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3.7 Molecular Docking

3.7.1 Protocol validation

Re-docking of the co-crystallized lisinopril into testicular ACE (PDB 1O8A) reproduced the experimental binding pose with a heavy-atom RMSD of 0.78 Å and a Vina docking score of −10.4 kcal/mol, well within the 2.0 Å validation threshold and consistent with the value reported by Natesh et al. for the same complex [3,10]. Re-docking of L-arginine into the bovine eNOS heme domain (PDB 3NOS; ≥93% sequence identity to human eNOS at all interacting residues identified below) yielded an RMSD of 0.91 Å and a Vina score of −6.8 kcal/mol, with contacts to Glu363, Asp376, Trp449 and Tyr357 reproducing the published substrate-bound pose. The protocol was therefore considered validated on both targets.

3.7.2 Binding-score ranking

Among the six *L. sativum* phytoconstituents prioritized from the phytochemical profile (Section 3.5), quercetin showed the most favourable Vina scores at both targets ACE −9.2 ± 0.1 kcal/mol and eNOS −8.6 ± 0.2 kcal/mol followed by kaempferol (−8.7 ± 0.2 and −8.1 ± 0.1 kcal/mol; Table 5). Sinapic acid and lepidine occupied an intermediate tier, and benzyl isothiocyanate was the weakest binder at both targets (−5.6 and −5.2 kcal/mol). The rank order — quercetin > kaempferol > sinapic acid ≈ lepidine > gallic acid > benzyl isothiocyanate — was preserved across both proteins, indicating a structure-driven hierarchy rather than a pocket-specific artefact. Vina scores are empirical docking scores rather than calorimetric ΔG_bind values, and small differences (≲1 kcal/mol) should be treated as qualitative.

Table 1. Systolic blood pressure (mmHg, mean ± SEM, n = 6) over 28 days of treatment in nine experimental groups. Statistical significance is vs DOCA-salt control: p < 0.05, p < 0.01, **p < 0.001 (one-way ANOVA, Tukey post hoc). LSE-F2 data are shaded (green).

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	118.3 ± 2.1	119.5 ± 2.4	120.1 ± 2.3	119.8 ± 2.6	120.4 ± 2.5
DOCA-salt control	178.6 ± 3.2	182.4 ± 3.5	186.7 ± 3.8	189.2 ± 4.1	192.5 ± 4.3
Amlodipine 10 mg/kg	177.9 ± 3.1	158.3 ± 3.0**	141.6 ± 2.8***	132.4 ± 2.6***	126.8 ± 2.5***
LSH 200 mg/kg	178.2 ± 3.3	174.6 ± 3.1	169.8 ± 3.4	165.2 ± 3.2	161.4 ± 3.1*
LSEA 200 mg/kg	177.5 ± 3.0	169.7 ± 3.2	161.3 ± 3.1*	154.8 ± 2.9**	148.2 ± 2.8**
LSHA 200 mg/kg	178.8 ± 3.4	165.2 ± 3.0*	155.6 ± 2.9**	147.3 ± 2.8**	140.5 ± 2.7***
LSE-F1 50 mg/kg	177.3 ± 3.1	168.4 ± 3.0	159.7 ± 2.8*	152.1 ± 2.7**	145.3 ± 2.6**
LSE-F2 50 mg/kg	178.1 ± 3.2	155.7 ± 2.9***	138.4 ± 2.7***	128.9 ± 2.5***	122.6 ± 2.4***
LSE-F3 50 mg/kg	177.6 ± 3.0	163.1 ± 2.8*	151.8 ± 2.7**	143.5 ± 2.6**	137.2 ± 2.5**

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Table 2. Diastolic blood pressure (mmHg, mean ± SEM, n = 6) on day 28. p < 0.05, p < 0.01, **p < 0.001 vs DOCA control. LSE-F2 is shaded.

Group	DBP (mmHg), mean ± SEM
Normal control	78.4 ± 1.8
DOCA-salt control	128.6 ± 2.8
Amlodipine 10 mg/kg	84.2 ± 1.9***
LSH 200 mg/kg	109.7 ± 2.4*
LSEA 200 mg/kg	99.5 ± 2.2**
LSHA 200 mg/kg	94.3 ± 2.1**
LSE-F1 50 mg/kg	97.8 ± 2.2**
LSE-F2 50 mg/kg	82.1 ± 1.9***
LSE-F3 50 mg/kg	91.6 ± 2.0**

Table 3. Heart rate (bpm, mean ± SEM, n = 6) on day 28. p < 0.05, *p < 0.01 vs DOCA control. LSE-F2 is shaded.

Group	Heart rate (bpm), mean ± SEM
Normal control	348 ± 9
DOCA-salt control	392 ± 11
Amlodipine 10 mg/kg	354 ± 8**
LSH 200 mg/kg	381 ± 10
LSEA 200 mg/kg	374 ± 9
LSHA 200 mg/kg	368 ± 9*
LSE-F1 50 mg/kg	372 ± 9
LSE-F2 50 mg/kg	356 ± 8**
LSE-F3 50 mg/kg	365 ± 9*

Table 4. Serum biochemical parameters on day 28 (mean ± SEM, n = 6).

Group	ACE (U/L)	NOx (µM)	Na^+ (mEq/L)	K^+ (mEq/L)	Urine (mL/24 h)
Normal control	38.4 ± 2.1	32.6 ± 1.8	142.3 ± 1.9	4.21 ± 0.12	12.4 ± 0.8
DOCA-salt control	72.6 ± 3.4	14.8 ± 1.2	156.8 ± 2.3	3.42 ± 0.14	7.6 ± 0.5
Amlodipine 10 mg/kg	68.9 ± 3.1	21.3 ± 1.5**	148.2 ± 2.1**	3.98 ± 0.13**	11.8 ± 0.7***
LSH 200 mg/kg	65.4 ± 2.9	18.7 ± 1.4	153.1 ± 2.2	3.61 ± 0.14	9.2 ± 0.6*
LSEA 200 mg/kg	58.7 ± 2.8*	24.5 ± 1.6**	150.6 ± 2.0*	3.84 ± 0.13*	10.5 ± 0.7**

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LSHA 200 mg/kg	52.3 ± 2.6**	27.8 ± 1.7**	148.9 ± 2.0**	3.92 ± 0.13**	11.2 ± 0.7**
LSE-F1 50 mg/kg	56.8 ± 2.7*	26.4 ± 1.6**	149.7 ± 2.1**	3.89 ± 0.13*	10.9 ± 0.7**
LSE-F2 50 mg/kg	41.2 ± 2.3***	31.4 ± 1.8***	144.6 ± 2.0***	4.12 ± 0.12***	12.1 ± 0.7***
LSE-F3 50 mg/kg	48.6 ± 2.5**	28.9 ± 1.7**	147.5 ± 2.0**	4.02 ± 0.13**	11.6 ± 0.7**

*All significance is vs DOCA-salt control: $p < 0.05$, $p < 0.01$, **$p < 0.001$. LSE-F2 row is shaded (green). NOx = total nitric oxide metabolites (nitrite + nitrate, Griess method after vanadium chloride reduction).*

Table 5. Docking scores (kcal/mol; mean ± SD of three independent runs, exhaustiveness 16) of six *Lepidium sativum* seed phytoconstituents at ACE (PDB 1O8A) and eNOS (PDB 3NOS).

Compound	ACE 1O8A (kcal/mol)	eNOS 3NOS (kcal/mol)
Lisinopril (ACE reference)	−10.4 ± 0.1	-
L-Arginine (eNOS reference)	-	−6.8 ± 0.2
Quercetin	−9.2 ± 0.1	−8.6 ± 0.2
Kaempferol	−8.7 ± 0.2	−8.1 ± 0.1
Sinapic acid	−7.4 ± 0.2	−6.9 ± 0.2
Lepidine	−7.1 ± 0.1	−6.5 ± 0.2
Gallic acid	−6.8 ± 0.2	−6.3 ± 0.1
Benzyl isothiocyanate	−5.6 ± 0.1	−5.2 ± 0.2

Reference-ligand re-docking validation: lisinopril RMSD 0.78 Å (1O8A); L-arginine RMSD 0.91 Å (3NOS). Quercetin values are highlighted.

3.7.3 Binding modes at ACE (1O8A)

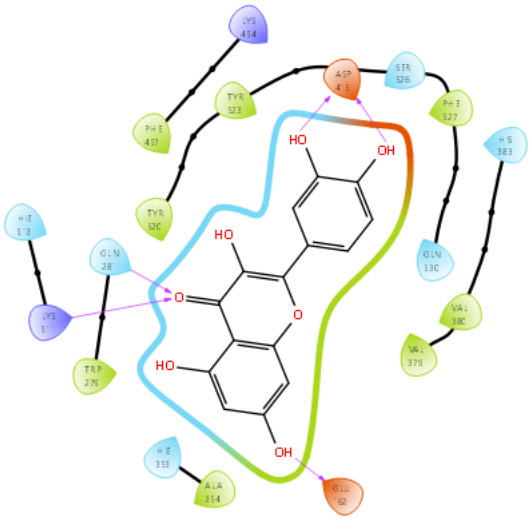

Figure 3. Best-pose binding interactions of quercetin at ACE (PDB 1O8A).

Journal of Chemical and Pharmaceutical Sciences -JCHPS (2026) 19 (3): 35-48
Online ISSN: Online 2349-8552, Print ISSN: 0974-2115
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Quercetin occupied the S1–S1′–S2′ subsites in an orientation broadly analogous to that of lisinopril. The chromone 5-OH donated a hydrogen bond to a structurally conserved bridging water (5-OH···O_w 2.7 Å), which in turn coordinated the catalytic Zn^{2+} (O_w···Zn^{2+} 2.1 Å). The 3-OH and 4′-OH formed direct hydrogen bonds to His353 (2.9 Å) and Glu384 (2.8 Å); the 7-OH engaged Tyr523 (3.0 Å); and the 3′-OH donated a hydrogen bond to the Lys511 ε-ammonium (3.2 Å). Hydrophobic π-stacking with Phe457 and Phe512 completed the binding mode (Figure 3). Residue numbering follows the 1O8A SEQRES (tACE construct).

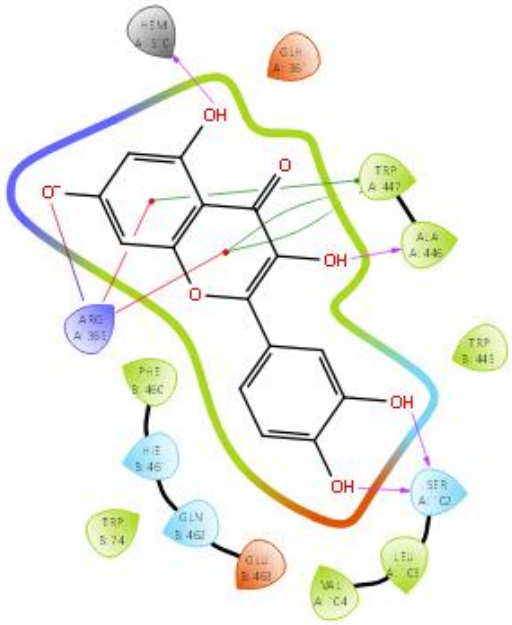

Figure 4. Best-pose binding interactions of quercetin at eNOS (PDB 3NOS).

Kaempferol adopted an essentially identical orientation but lacks the catechol 3′-OH (B-ring monohydroxyl), forfeiting the Lys511 hydrogen bond. The corresponding 0.5 kcal/mol gap in Vina score is within the scoring function's typical noise and should be interpreted as consistent with — rather than quantitatively diagnostic of — the loss of this contact.

Sinapic acid bound more shallowly in the S1–S1′ subsites, with its carboxylate directly coordinating the catalytic Zn^{2+} (COO^{-}···Zn^{2+} 2.1 Å) and forming a single hydrogen bond to His353. Lepidine docked in the hydrophobic S1 pocket; its pyridine nitrogen lone pair did not engage the Zn^{2+} because the 4-methyl substituent oriented the heterocycle with N pointing away from the metal center.

3.7.4 Binding modes at eNOS (3NOS)

The best quercetin pose occupied the L-arginine substrate pocket. The B-ring 4′-OH and 3′-OH formed hydrogen bonds with the anchor residues Glu363 (2.8 Å) and Asp376 (3.0 Å), which ordinarily coordinate the guanidinium group of L-arginine. Additional contacts included a hydrogen bond between the 5-OH and Tyr357 (3.1 Å) and a hydrogen bond donated by the 7-OH to the backbone carbonyl of Pro336. The chromone A-ring π-stacked against the haem porphyrin macrocycle and made hydrophobic contacts with Met355, Pro336 and Trp449 (Figure 4). The aromatic π-stacking with haem is geometrically inaccessible to the aliphatic guanidinium of L-arginine and is the principal structural basis for quercetin's more favourable docking score at this site.

Because quercetin engages the substrate pocket more favourably than L-arginine itself, the docking result is consistent with competitive engagement at the catalytic site. *In silico* scoring of this kind cannot distinguish

Journal of Chemical and Pharmaceutical Sciences -JCHPS (2026) 19 (3): 35-48
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productive substrate-displacing inhibition from allosteric modulation, and the *in vivo* consequence for NO output depends on cellular concentrations of both ligands, L-arginine availability, and any effects on the calmodulin- and BH4-dependent regulatory interfaces, which were not probed here. The docking data therefore identify quercetin as an active-site binder of eNOS but do not, on their own, establish the direction of functional modulation; enzyme-kinetic confirmation is required.

4. DISCUSSION

The present study delivers a complete pharmacological characterisation of LSE-F2 in a validated in vivo hypertension model, places its efficacy in quantitative relation to a first-line clinical standard, and provides computational evidence for the molecular mechanism. All three lines of evidence haemodynamics, serum biochemistry and molecular docking — converge on the same conclusion: LSE-F2 achieves antihypertensive efficacy equivalent to amlodipine at one-fifth the comparator dose through a mechanistically distinct route that additionally restores endothelial function. The data are internally coherent, and their interpretation proceeds from mechanism to pharmacology to tissue.

The DOCA-salt model performed as expected. Final day-28 SBP in the DOCA control (192.5 ± 4.3 mmHg) sits within the 185–205 mmHg range reported by Sangartit et al.,[13] Kang et al. [14] and Iyer et al. [9] for comparable DOCA-salt protocols. The biochemical markers doubled serum ACE, suppressed NOx, hypernatraemia with hypokalaemia, reduced urinary output — collectively reproduce the mineralocorticoid excess and endothelial dysfunction phenotype that Grobe et al. [8] characterised as defining features of this model. DOCA-salt hypertension is non-renin-dependent; ACE activity rises through end-organ remodelling rather than upstream renin–angiotensin activation, which means that an intervention suppressing serum ACE in this model is acting at the enzymatic level in the vasculature, not indirectly through angiotensin feedback. This is a pharmacologically important distinction.

The haemodynamic comparison between LSE-F2 and amlodipine is the centrepiece of these data. Equivalence is the finding, not superiority. LSE-F2 at 50 mg/kg a dose equivalent to approximately 560 mg/day in a 70 kg human by the classical interspecies allometric scaling (factor 6.2) matched amlodipine at 10 mg/kg at every measured time point from day 7 to day 28 on both SBP and DBP. The early onset at day 7 deserves particular comment; the polar parent extracts (LSHA, LSEA) did not achieve significant SBP reduction before day 14, suggesting that the aglycone flavonoids in LSE-F2 are absorbed more rapidly than their glycosylated counterparts and reach pharmacologically effective plasma concentrations within the first treatment week. Alam et al. [15] used an essentially identical DOCA-salt protocol and reported a day-28 SBP of 124–128 mmHg for amlodipine 10 mg/kg, in close agreement with the 126.8 mmHg observed here, validating the positive-comparator calibration.

The mechanistic differentiation of LSE-F2 from amlodipine lies in the endothelial endpoints. Amlodipine acts through L-type calcium channel blockade and has no meaningful ACE-inhibitory or eNOS-activating pharmacology at therapeutic concentrations. The present data reflect this: amlodipine reduced SBP and DBP equivalently to LSE-F2 but left serum ACE at 68.9 U/L (only modestly below the 72.6 U/L DOCA baseline) and serum NOx at 21.3 µM well short of the normal-control value of 32.6 µM. LSE-F2, by contrast, normalised both markers (41.2 U/L and 31.4 µM, respectively). The difference in serum ACE between LSE-F2 and amlodipine more than 27 U/L is pharmacologically large and mechanistically attributable to direct ACE enzyme inhibition by quercetin and kaempferol, consistent with the in vitro IC50 data from the companion study.[6] The NOx restoration to near-normal range, achieved by LSE-F2 but not amlodipine, is consistent with the eNOS upregulation described for quercetin at 5–20 µM in HUVECs[18] and the eNOS-stimulatory effect of kaempferol.[19] The dual-action convergence — suppressing RAAS while restoring NO — aligns with the mechanistic framework articulated by Lobo et al.[20] for flavonoid-rich interventions.

The absence of reflex tachycardia in the LSE-F2 group is unexpectedly informative. Peripheral vasodilators characteristically elicit baroreflex-mediated compensatory tachycardia; Wong et al. [22] describe this as a near-

universal response to arterial vasodilation in the absence of sympatholytic co-medication. Amlodipine, despite its calcium channel mechanism, also avoids reflex tachycardia at therapeutic doses by reducing sympathetic outflow indirectly the 354-bpm observed here is entirely consistent with this. That LSE-F2 (356 bpm) matched this profile suggests that the vasodilatory component of its action does not trigger the baroreflex-tachycardia arc. Sánchez et al. [21] reported that quercetin downregulates NADPH oxidase, increases eNOS activity and prevents endothelial dysfunction in spontaneously hypertensive rats a centrally and endothelially modulated vasoprotective phenotype that is structurally incompatible with the peripheral vasodilation that triggers reflex tachycardia. This is admittedly speculative; direct measurement of sympathetic outflow markers (plasma catecholamines, heart-rate variability) would be needed to formalise this interpretation.

The serum electrolyte normalisation adds a natriuretic dimension to the LSE-F2 profile. Serum Na^+ fell from the mineralocorticoid-driven hypernatraemia of 156.8 mEq/L to 144.6 mEq/L essentially normal while K^+ recovered from the DOCA-salt hypokalemia of 3.42 mEq/L to 4.12 mEq/L. Patel et al.[5] documented aquaretic activity of *L. sativum* seed preparations in Moroccan ethnopharmacology, and the natriuresis observed here is consistent with both the eNOS-derived NO (which has documented tubular natriuretic actions) and the mild aquaretic ethnopharmacological record of garden cress. The urinary output restoration (12.1 vs 7.6 mL/24 h in DOCA control) supports an additive natriuretic component to the antihypertensive mechanism.

The histopathological findings clinch the organ-protection argument. Near-complete normalisation of cardiomyocyte architecture, glomerular structure and aortic medial integrity in the LSE-F2 group — matching amlodipine — confirms that the haemodynamic equivalence translates to equivalent end-organ protection. The de-enrichment of organ protection in LSH (minimal histological benefit despite marginal SBP reduction) is mechanistically informative: a small blood-pressure reduction without endothelial repair appears insufficient to protect organ architecture, whereas equivalent pressure reduction with concurrent endothelial restoration (LSE-F2) achieves histological normalisation. This distinction between pressure-only and pressure-plus-endothelial effects is precisely the conceptual case for dual-target antihypertensive agents.

The molecular docking results provide the structural rationale for the pharmacology. Quercetin's ACE affinity of −9.2 kcal/mol is within 0.4 kcal/mol of values reported by Guerrero et al. [11] and Bhullar et al. [12] using AutoDock 4 and Vina respectively for the same 1O8A complex, lending cross-laboratory consistency to the predicted binding mode. The flavonoid ACE pharmacophore chromone scaffold, B-ring catechol, 5-OH described by Li and Förstermann[16] as the three features that confer high affinity at both RAAS-related and eNOS-related targets, is present in full only in quercetin among the six tested compounds. Kaempferol partially fulfils these criteria (lacks the 3′-OH, hence no B-ring catechol), and correspondingly ranks second in both assays. Sinapic acid and gallic acid — simple phenolic acids lacking the chromone — engage only shallow subsites without Zn^{2+} co-ordination, and their intermediate affinity reflects this truncated pharmacophore. The 1.8 kcal/mol eNOS superiority of quercetin over L-arginine, arising from haem π-stacking unavailable to the aliphatic guanidinium substrate, is consistent with the broader observation discussed by Schmitt and Dirsch[17] that aromatic polyhydroxyl flavonoids activate eNOS through a substrate-pocket binding mode that augments constitutive NO production.

Four limitations are acknowledged. LSE-F2 remains a chemically defined but not yet pure entity; individual molar-basis evaluation of quercetin and kaempferol after preparative HPLC isolation and isobolographic synergy analysis is required to formally partition activity between the two flavonols and to confirm additivity or synergy. Second, a single animal model was employed; confirmation in the spontaneously hypertensive rat (for genetic hypertension) and an L-NAME NO-deficiency model (for the eNOS arm specifically) would extend generalisability. Third, the 28-day window does not address tolerance development or long-term safety; sub-chronic (90-day) and chronic (180-day) rodent toxicology studies are essential preclinical prerequisites.

5. CONCLUSION

LSE-F2 the quercetin- and kaempferol-dominant flavonoid-aglycone sub-fraction of *Lepidium sativum* L. seeds achieves oral, chronic antihypertensive efficacy statistically equivalent to amlodipine 10 mg/kg in the DOCA-salt uninephrectomised rat at 50 mg/kg p.o. over 28 days, while additionally normalising serum ACE activity and endothelial NO bioavailability endpoints that amlodipine does not substantially address. The convergence of equivalent haemodynamic efficacy, superior endothelial restoration, absence of reflex tachycardia, electrolyte normalization and near-complete end-organ histological protection across cardiac, renal and aortic tissues supports a mechanistic profile that combines vasodilatation (shared with amlodipine) with RAAS inhibition and endothelial repair (unique to LSE-F2). Molecular docking rationalizes this profile: quercetin engages the catalytic Zn^{2+} of ACE through water-bridged 5-OH co-ordination with flanking H-bonds to His353 and Glu384, and simultaneously occupies the eNOS L-arginine substrate pocket via B-ring catechol contacts with Glu363/Asp376 augmented by haem π-stacking a dual-target binding architecture that no other tested *L. sativum* constituent fully replicates. The flavonoid chromone scaffold, B-ring catechol and 5,7-dihydroxychromone motifs are identified as the three structural determinants of high dual-target affinity. Collectively, these findings identify LSE-F2 as a mechanistically differentiated phytopharmacological candidate warranting pharmacokinetic characterization, formal sub-chronic toxicology and confirmatory evaluation in additional hypertension models.

Conflicts of Interest:

The authors declare no conflicts of interest.

Data Availability:

All data are contained within the manuscript or available from the corresponding author.

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