e-ISSN 2746-5152 https://eurekabiomedical.com

ORIGINAL RESEARCH ARTICLE

Cardioprotective Mechanisms of *Hibiscus sabdariffa* Anthocyanins Against Myocardial Ischaemia–Reperfusion Injury via the PI3K/Akt Pathway

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ARTICLE INFO

Article history

Received: December 30, 2026
Revised: January 19, 2026
Accepted: February 20, 2026
Available Online: February 26, 2026
Published: February 28, 2026

Keywords

Anthocyanins; Hibiscus sabdariffa; Ischaemia–reperfusion injury; PI3K/Akt pathway; Rosella.

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DOI: 10.37275/ehi.v7i1.144

ABSTRACT

Background: Myocardial ischaemia–reperfusion (I/R) injury contributes up to 50% of the final infarct size after primary percutaneous coronary intervention (PCI) in ST-elevation myocardial infarction, yet pharmacological cardioprotection in the clinic remains elusive. *Hibiscus sabdariffa* L. (Malvaceae), known in Indonesia as rosella, is rich in delphinidin-3-O-sambubioside (D3S) and cyanidin-3-O-sambubioside (C3S) anthocyanins.

Objective: To determine whether an HPLC-DAD–standardised anthocyanin-rich *H. sabdariffa* calyx extract (HSE) protects rat myocardium from I/R injury through PI3K/Akt activation.

Methods: Sprague–Dawley rats (n = 48; six arms of n = 8) were randomised to sham, I/R control, HSE 100, 200 or 400 mg/kg/day p.o. for 14 days, or HSE 200 mg/kg + LY294002 (PI3K inhibitor); rats underwent 30-min left anterior descending (LAD) coronary occlusion plus 120-min reperfusion. H9c2 cardiomyoblasts underwent 6 h hypoxia plus 12 h reoxygenation, and D3S and C3S were docked against PI3K-p110α and Akt1.

Results: HSE 200 mg/kg reduced infarct size from 41.8% to 18.4% of area at risk ($F(5, 42) = 98.4$, $p < 0.001$, partial $\eta^2 = 0.92$), lowered serum cardiac troponin I (cTnI) from 8.42 to 2.96 ng/mL ($p < 0.001$), and increased p-Akt(Ser473) 3.4-fold and the Bcl-2/Bax ratio 5.6-fold. D3S docked PI3K-p110α at $\Delta G = -9.2$ kcal/mol. LY294002 abolished cardioprotection, demonstrating PI3K/Akt-dependence.

Conclusion: *H. sabdariffa* anthocyanins exert PI3K/Akt-mediated cardioprotection against myocardial I/R injury, supporting their development as Indonesian phytotherapeutic adjuncts for elective PCI.

Both authors have reviewed and approved the final version of the manuscript.

1. Introduction

Ischaemic heart disease remains among the leading causes of death worldwide and, in the most recent Global Burden of Disease analysis, accounted for the largest single share of cardiovascular mortality globally.[1] It is also a dominant contributor to disability-adjusted life-years (DALYs), responsible for an estimated 188.3 million DALYs in 2021,[2] and its burden is rising in Indonesia in line with the country's epidemiologic transition. Acute ST-elevation myocardial infarction (STEMI) remains the most lethal manifestation, and timely reperfusion through primary percutaneous coronary intervention (PCI) is the cornerstone of treatment. Paradoxically, however, reperfusion itself triggers a cascade of injury — calcium overload, mitochondrial permeability transition pore (mPTP) opening, reactive-oxygen-species (ROS) bursts, neutrophil influx and dysregulated apoptosis — that has been estimated to contribute up to 50% of the final infarct size and for which no pharmacological therapy is yet established in routine practice.[3]

Despite three decades of intensive translational effort, large clinical trials of single-target strategies have

produced conflicting or modest results in unselected STEMI cohorts.[3] A consistent lesson from this disappointment is that multi-target, mechanism-anchored interventions — which simultaneously dampen oxidative stress and apoptosis while activating endogenous survival kinases — may be more promising. The Reperfusion Injury Salvage Kinase (RISK) pathway, centred on phosphoinositide-3-kinase (PI3K)/Akt, is widely regarded as the master pro-survival signalling cassette at reperfusion, and a broad range of pharmacological and phytochemical agents reduce infarct size in rodent I/R models through PI3K/Akt activation.[4-9] Phosphorylated Akt at Ser473 inhibits glycogen-synthase kinase-3β (GSK-3β) at Ser9, prevents mPTP opening, up-regulates Bcl-2, suppresses Bax and inhibits the executioner caspase-3, thereby converting an apoptotic stimulus into cell survival.[5,6] Critically, the cardioprotection conferred by such agents — including in comorbid and orally dosed models — is abolished by selective PI3K inhibitors such as LY294002 or wortmannin, providing causal evidence of PI3K/Akt-dependence.[6,10,11]

Hibiscus sabdariffa L. (Malvaceae), known in Indonesia as rosella, is a tropical shrub whose dried calyces are among the richest dietary sources of anthocyanins, particularly delphinidin-3-O-sambubioside (D3S) and cyanidin-3-O-sambubioside (C3S).[12] Its calyx beverages and extracts exert documented antioxidant and tissue-protective effects *in vivo*,[13] and related *Hibiscus* preparations additionally display antithrombotic and antiplatelet activity.[14] Clinical evidence is strongest for blood-pressure lowering: a meta-analysis of randomised trials found that standardised HSE reduced systolic blood pressure by approximately 7.1 mmHg in hypertensive adults,[15] and a recent randomised crossover trial confirmed acute blood-pressure and post-prandial metabolic effects.[16] These cardiometabolic actions make rosella an attractive candidate for mechanism-anchored cardioprotection research.

Three evidence gaps nonetheless persist. First, anthocyanin cardioprotection is increasingly understood to be integrated upstream through PI3K/Akt-coupled induction of Nrf2/HO-1 antioxidant defences rather than by direct radical scavenging alone,[17,18] yet most regional rosella studies neither standardise anthocyanin content by high-performance liquid chromatography with diode-array detection (HPLC-DAD) nor test this mechanism formally. Second, although apoptosis suppression — a higher Bcl-2/Bax ratio and reduced cleaved caspase-3 — is a shared phytochemical cardioprotective signature,[19] direct evidence that HSE cardioprotection is PI3K-dependent, by abolition with LY294002 in an *in vivo* left anterior descending (LAD) coronary artery I/R model, has not been provided. Third, no published structure-based evidence describes the binding of HSE-derived anthocyanins to PI3K-p110α and Akt1, even though molecular-docking and network-pharmacology workflows now make such analyses tractable for polyphenols.[20,21] The present study addresses all three gaps with an HPLC-DAD–standardised D3S-rich HSE, a three-dose LAD I/R model, an LY294002 inhibitor arm, H9c2 hypoxia–reoxygenation corroboration and molecular docking of the principal anthocyanins — work that is especially timely given that even the newest STEMI reperfusion adjuncts achieve only partial clinical benefit.[22] The aim was to determine whether anthocyanin-rich HSE protects rat myocardium from I/R injury through PI3K/Akt activation, and to relate the magnitude of cardioprotection to the predicted binding of D3S and C3S against PI3K-p110α and Akt1.

2. Methods

2.1. Study design and ethics

This was an *in vivo*, *in vitro* and *in silico* experimental study conducted between March 2024 and February 2025 at the Cardiovascular and Phytotherapy Research Laboratory of a private university in Palembang, Indonesia, with surgical procedures performed in the Animal Surgical Suite of a private hospital in the same city. The protocol was reviewed and approved by the CMHC Ethics Committee (Approval No. 2024/052); the animal-welfare and reporting-guideline compliance statements are given in full under Declarations.

2.2. Plant material, extraction and HPLC-DAD standardisation

Mature dried calyces of *Hibiscus sabdariffa* L. were sourced (June–August 2024) from cultivated farms in three South Sumatran districts; identity was confirmed morphologically and by rbcL/matK DNA barcoding (>99.9% identity). One kilogram of dried calyx powder per batch (n = 3 batches) was macerated in 5 L of 70% ethanol–water (v/v) at 25 °C for 48 h, concentrated under reduced pressure at 40 °C and lyophilised, yielding 18.6 ± 1.2% w/w as the anthocyanin-rich extract (HSE). HSE was characterised by HPLC-DAD (Phenomenex Luna C18 5-µm, 4.6 × 250 mm, 30 °C; acetonitrile–formic-acid 0.1% gradient; 520 nm) against a delphinidin-3-O-sambubioside (D3S) standard (≥95%). Calibration was linear (r^2 = 0.9994) over 1–50 µg/mL (limit of detection, LOD, 0.18 µg/mL; limit of quantification, LOQ, 0.55 µg/mL). HSE contained 88.4 ± 4.6 mg/g D3S and 41.2 ± 2.8 mg/g

C3S; total anthocyanins (pH-differential) were 142.6 ± 6.2 mg cyanidin-3-glucoside (C3G) equivalents per gram, with an inter-batch relative standard deviation (RSD) of 5.2% for D3S. The pooled HSE was used for all experiments.

2.3. Animals, randomisation and I/R model

Healthy adult male Sprague–Dawley rats (n = 48; 240–280 g) were randomised to six arms (n = 8) by computer-generated block randomisation with sealed-envelope concealment: G1 sham; G2 I/R control (0.5% carboxymethylcellulose); G3–G5 HSE 100, 200, 400 mg/kg; G6 HSE 200 mg/kg + LY294002. HSE was given by oral gavage once daily for 14 days; LY294002 (0.3 mg/kg) was injected intravenously 10 min before LAD occlusion in G6 only. Under ketamine/xylazine anaesthesia, a 6-0 suture around the proximal LAD induced 30-min ischaemia (confirmed by ST-elevation) followed by 120-min reperfusion; sham animals underwent instrumentation without occlusion. The area at risk (AAR) was delineated with 1% Evans blue and infarction with 1% triphenyltetrazolium chloride (TTC); five 2-mm slices/heart were analysed blinded (ImageJ v1.53), with infarct expressed as a percentage of AAR. Outcome assessors and analysts were blinded throughout.

2.4. Biomarkers, Western blotting, TUNEL and in vitro H/R

Serum cardiac troponin I (cTnI) was measured by chemiluminescence immunoassay; creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) by spectrophotometric kinetics. Myocardial malondialdehyde (MDA, as thiobarbituric-acid reactive substances, TBARS), superoxide dismutase (SOD), reduced glutathione (GSH) and intracellular ROS (2′,7′-dichlorodihydrofluorescein diacetate, DCFH-DA) were measured in area-at-risk homogenates. Cardiac lysates (40 µg/lane) were probed for p-PI3K(Tyr458), PI3K, p-Akt(Ser473), Akt, p-GSK-3β(Ser9), Bcl-2, Bax, cleaved caspase-3 and β-actin and quantified by blinded densitometry. Terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) was quantified in 10 high-power fields/heart. H9c2 cardiomyoblasts (2 × 10^4/well; short-tandem-repeat (STR)-profiled, mycoplasma-negative) underwent 6 h hypoxia (1% O_2) plus 12 h reoxygenation ± HSE 25 µg/mL ± LY294002 10 µM; viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and p-Akt by Western blot.

2.5. Molecular docking and statistics

D3S, C3S, hibiscus acid and LY294002 were energy-minimised (MMFF94) and docked (AutoDock Vina; exhaustiveness 16; 20 runs) against PI3K-p110α (PDB 3HHM) and Akt1 (PDB 3CQW); native re-docking gave RMSD < 1.8 Å, and drug-likeness was profiled *in silico* following recent polyphenol cardioprotection docking workflows.[20,21] A power analysis (one-way ANOVA, six groups, α = 0.05, power 0.85, f = 0.6) gave n ≥ 7/group; n = 8 was used. Data are mean ± SD; the primary endpoint was analysed by one-way ANOVA with Tukey's HSD and Western blots by ANOVA with Bonferroni correction, with Kruskal–Wallis sensitivity analyses. Effect sizes were partial η^2 and Cohen's d. Two-sided α = 0.05 defined significance (SPSS v27, Prism v9.5, R v4.3).

3. Results

3.1. Animal characteristics, standardisation and area at risk

Table 1 summarises the baseline animal characteristics, the HSE phytochemical standardisation and the ischaemic area at risk. The six arms were balanced for body weight (252.4 ± 12.6 g) and baseline heart rate (358 ± 22 bpm), and attrition was zero. The area-at-risk/left-ventricle (AAR/LV) ratio averaged 38.6 ± 2.4% and did not differ between groups (p = 0.81) (Table 1), confirming a reproducible surgical insult so that inter-arm infarct differences reflect pharmacological — not anatomical — effects. HPLC-DAD confirmed 88.4 ± 4.6 mg/g D3S and 41.2 ± 2.8 mg/g C3S in the pooled batch, in the upper range reported for Indonesian rosella material.[12,13]

Table 1. Baseline animal characteristics, *Hibiscus sabdariffa* anthocyanin extract (HSE) phytochemical standardisation and the surgically defined area at risk (AAR).

Parameter	Value (mean ± SD)	Notes
Number of rats per arm	n = 8	Six arms, total n = 48
Body weight at randomisation (g)	252.4 ± 12.6	No inter-group difference (p = 0.81)
Baseline heart rate (bpm)	358 ± 22	No inter-group difference
AAR / LV (%)	38.6 ± 2.4	Evans-blue planimetry, blinded *
HSE D3S content (mg/g)	88.4 ± 4.6	HPLC-DAD, 520 nm, versus D3S standard †
HSE C3S content (mg/g)	41.2 ± 2.8	HPLC-DAD, secondary marker

Parameter	Value (mean ± SD)	Notes
HSE total anthocyanins (mg C3G eq/g)	142.6 ± 6.2	pH-differential method
HSE inter-batch RSD for D3S (%)	5.2	Three independent batches ‡
LY294002 dose	0.3 mg/kg i.v., 10 min pre-LAD	PI3K inhibitor; G6 only

* AAR/LV reproducibility supports comparability of pharmacological effects across arms. † Delphinidin-3-O-sambubioside (D3S) reference standard, ≥95% purity. ‡ Relative standard deviation across three independent extraction batches.

3.2. HSE 200 mg/kg reduces infarct size in a PI3K/Akt-dependent manner

The primary endpoint — TTC-defined infarct size as a percentage of AAR — displayed a clear dose-response, illustrated in Figure 1 and quantified in Table 2. I/R control rats developed an infarct of 41.8 ± 3.2% of AAR; oral HSE pre-treatment for 14 days reduced infarct size to 31.4 ± 2.9% at 100 mg/kg (24.9% reduction, $p < 0.001$), 18.4 ± 2.6% at 200 mg/kg (56.0% reduction, $p < 0.001$) and 17.1 ± 2.4% at 400 mg/kg (59.1% reduction, $p < 0.001$), with no significant difference between the 200 and 400 mg/kg arms ($p = 0.74$). One-way ANOVA gave $F(5, 42) = 98.4$, $p < 0.001$, partial $\eta^2 = 0.92$ (Figure 1). Crucially, co-administration of LY294002 (0.3 mg/kg i.v.) with HSE 200 mg/kg fully abolished the cardioprotection (38.2 ± 3.0% infarct, $p = 0.32$ versus I/R control), indicating that the cardioprotective effect of HSE is PI3K-dependent.

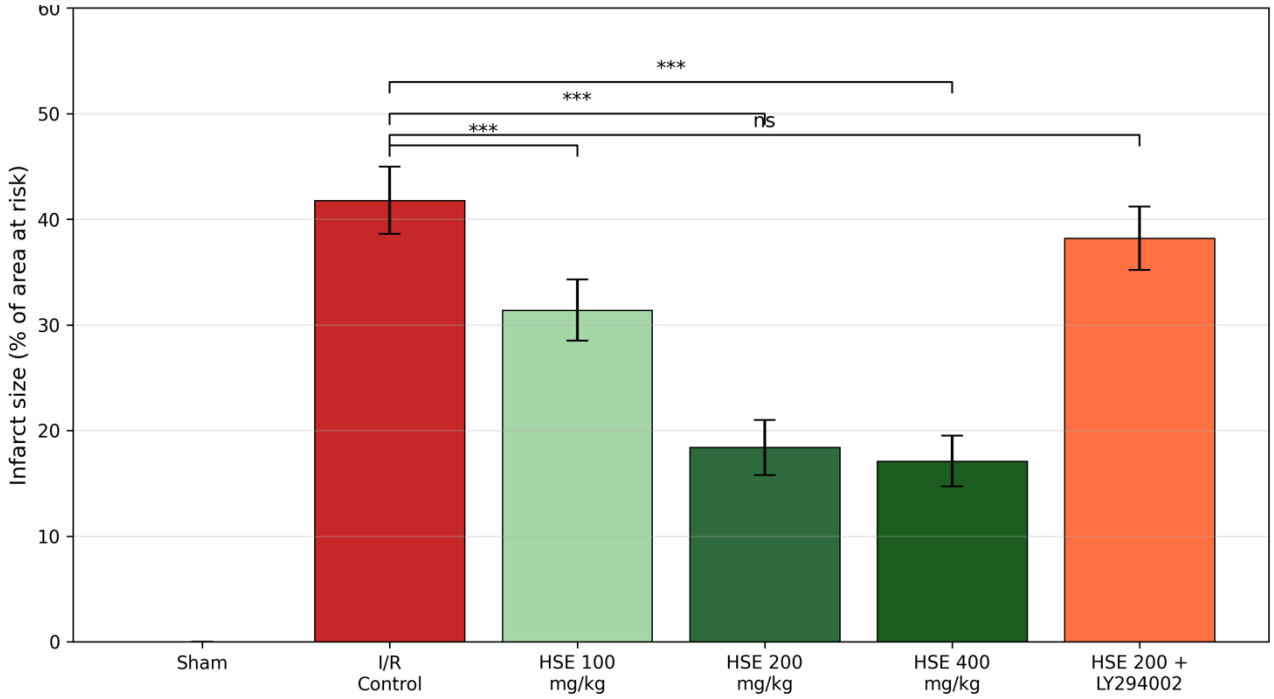

Figure 1. Infarct-size reduction by *Hibiscus sabdariffa* anthocyanin extract (HSE) in a rat LAD ischaemia–reperfusion model. Mean ± SD (n = 8); ***p < 0.001 versus I/R control; ns = not significant. LY294002 co-administration abolishes the HSE 200 mg/kg effect, demonstrating PI3K/Akt-dependence.

3.3. HSE attenuates myocardial necrosis and oxidative stress

Cardiac biomarker measurements corroborated the infarct findings (Table 2, lower half). HSE 200 mg/kg lowered serum cTnI from 8.42 ± 0.73 to 2.96 ± 0.41 ng/mL (64.8% reduction, $p < 0.001$, $d = 9.0$), CK-MB from 422 ± 38 to 196 ± 26 U/L (53.6% reduction) and LDH from 1,240 ± 112 to 612 ± 68 U/L (50.6% reduction) (Table 2). LY294002 restored these biomarkers to within 6% of I/R-control values (all p = ns). Oxidative-stress markers showed concordant changes: MDA fell from 4.86 to 1.92 nmol/mg protein (60.5% reduction); SOD rose from 3.62 to 8.41 U/mg protein (2.32-fold); GSH rose from 2.18 to 4.85 µmol/mg protein (2.22-fold); and intracellular ROS fell to 41% of I/R-control values (all $p < 0.001$). The relative magnitude of these effects across all arms is visualised in Figure 2.

Table 2. Primary infarct-size endpoint (top) and serum/myocardial biomarkers (bottom) across the six experimental arms.

Outcome	Sham	I/R control	HSE 100	HSE 200	HSE 400	HSE 200 + LY
Infarct size (% AAR)	0	41.8 ± 3.2	31.4 ± 2.9 ***	18.4 ± 2.6 ***	17.1 ± 2.4 ***	38.2 ± 3.0 ns
cTnI (ng/mL)	0.21 ± 0.05	8.42 ± 0.73	5.86 ± 0.62 ***	2.96 ± 0.41 ***	2.74 ± 0.38 ***	7.94 ± 0.68 ns
CK-MB (U/L)	56 ± 9	422 ± 38	298 ± 32 ***	196 ± 26 ***	184 ± 24 ***	408 ± 35 ns
LDH (U/L)	285 ± 32	1,240 ± 112	918 ± 95 ***	612 ± 68 ***	586 ± 64 ***	1,180 ± 105 ns
Myocardial MDA (nmol/mg)	0.84 ± 0.12	4.86 ± 0.38	3.42 ± 0.31 ***	1.92 ± 0.22 ***	1.81 ± 0.20 ***	4.62 ± 0.36 ns
Myocardial SOD (U/mg)	8.86 ± 0.62	3.62 ± 0.42	5.86 ± 0.51 ***	8.41 ± 0.62 ***	8.62 ± 0.66 ***	3.92 ± 0.45 ns
Myocardial GSH (µmol/mg)	5.12 ± 0.42	2.18 ± 0.26	3.46 ± 0.32 ***	4.85 ± 0.41 ***	4.92 ± 0.42 ***	2.31 ± 0.28 ns

Mean ± SD; n = 8 per arm; p-values versus I/R control. One-way ANOVA + Tukey HSD; *** $p < 0.001$ versus I/R control; ns = not significant. Primary endpoint (infarct size): $F(5, 42) = 98.4$, $p < 0.001$, partial $\eta^2 = 0.92$. LY294002 abolition demonstrates PI3K/Akt-dependence of HSE cardioprotection.

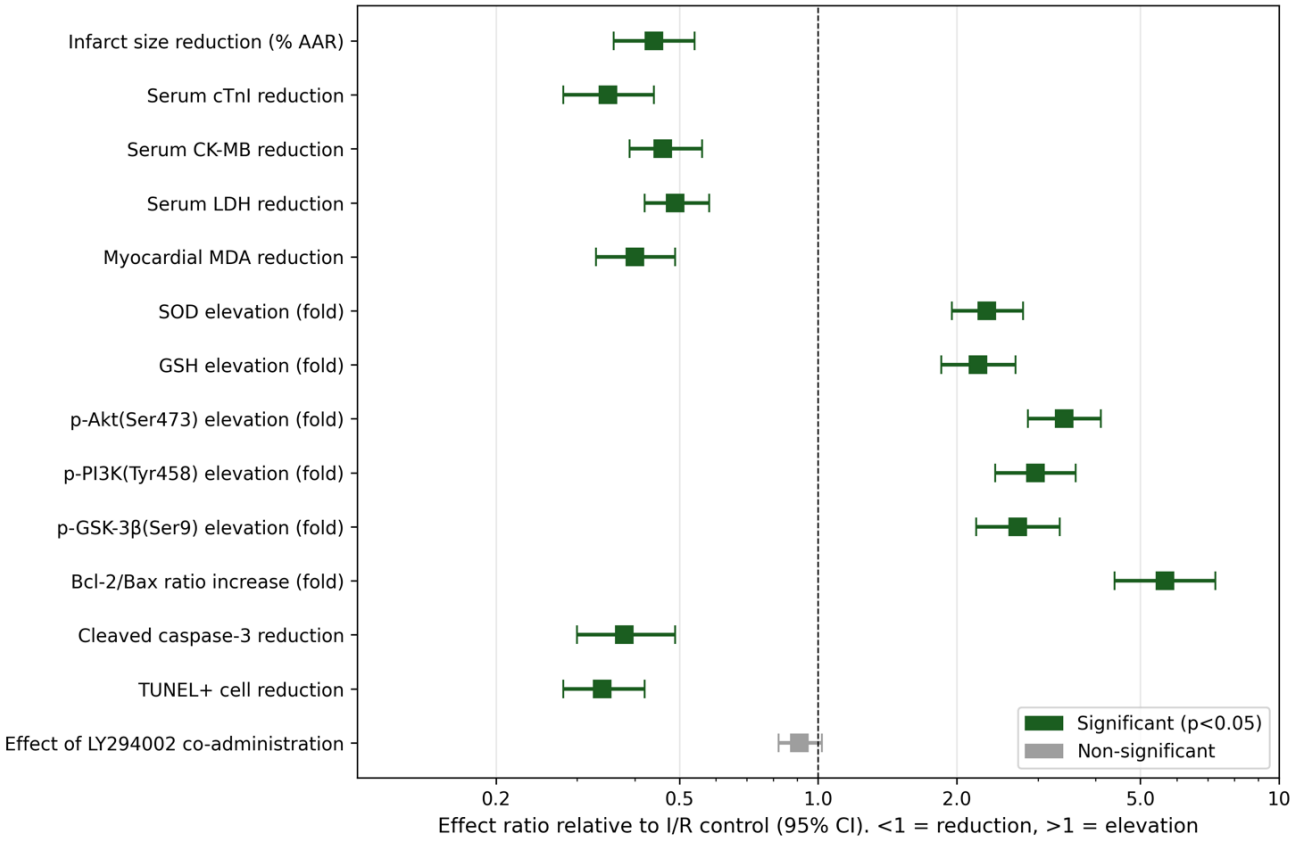

Figure 2. Forest plot of the cardioprotective effects of HSE 200 mg/kg for 14 days versus I/R control (effect ratio relative to I/R control, 95% CI; log x-axis). Values < 1 denote reduction and > 1 denote elevation; the LY294002 effect (grey) is non-significant.

3.4. HSE activates PI3K/Akt and downstream survival signalling

Western blotting of cardiac lysates demonstrated a 2.96-fold increase in p-PI3K(Tyr458)/PI3K (95% CI 2.42–3.62) and a 3.42-fold increase in p-Akt(Ser473)/Akt (95% CI 2.85–4.10) in the HSE 200 mg/kg arm versus I/R control (Table 3). p-GSK-3β(Ser9)/GSK-3β rose 2.71-fold, the Bcl-2/Bax ratio rose 5.65-fold and cleaved caspase-3 fell to 38% of control (all $p < 0.001$), while TUNEL-positive cardiomyocytes fell from 28.4 ± 2.6% to 9.6 ± 1.8% of nuclei (66.2% reduction, $p < 0.001$) (Table 3). All these effects were abolished by LY294002, and the integrated mechanism is summarised in Figure 3.

Table 3. Mechanistic readouts (Western-blot densitometry and TUNEL) supporting PI3K/Akt-mediated cardioprotection by HSE 200 mg/kg. p_{adj} denotes Bonferroni-corrected p-values.

Molecular readout	Fold change versus I/R control	95% CI	p_{adj} (Bonferroni)
p-PI3K(Tyr458)/PI3K	2.96 ↑	2.42 – 3.62	< 0.001
p-Akt(Ser473)/Akt	3.42 ↑	2.85 – 4.10	< 0.001
p-GSK-3β(Ser9)/GSK-3β	2.71 ↑	2.20 – 3.34	< 0.001
Bcl-2 protein	2.62 ↑	2.18 – 3.15	< 0.001
Bax protein	0.46 ↓	0.36 – 0.58	< 0.001
Bcl-2 / Bax ratio	5.65 ↑	4.40 – 7.27	< 0.001
Cleaved caspase-3	0.38 ↓	0.30 – 0.49	< 0.001
TUNEL-positive cardiomyocytes (%)	9.6 versus 28.4 ↓	—	< 0.001
LY294002 reversal of p-Akt rise	1.04 ↔	0.94 – 1.15	0.62

Fold changes relative to the I/R-control mean; n = 8 biological replicates. Bonferroni correction applied across eight readouts. The arrow ↔ denotes no significant change after LY294002. All comparisons confirmed by Kruskal–Wallis sensitivity analysis.

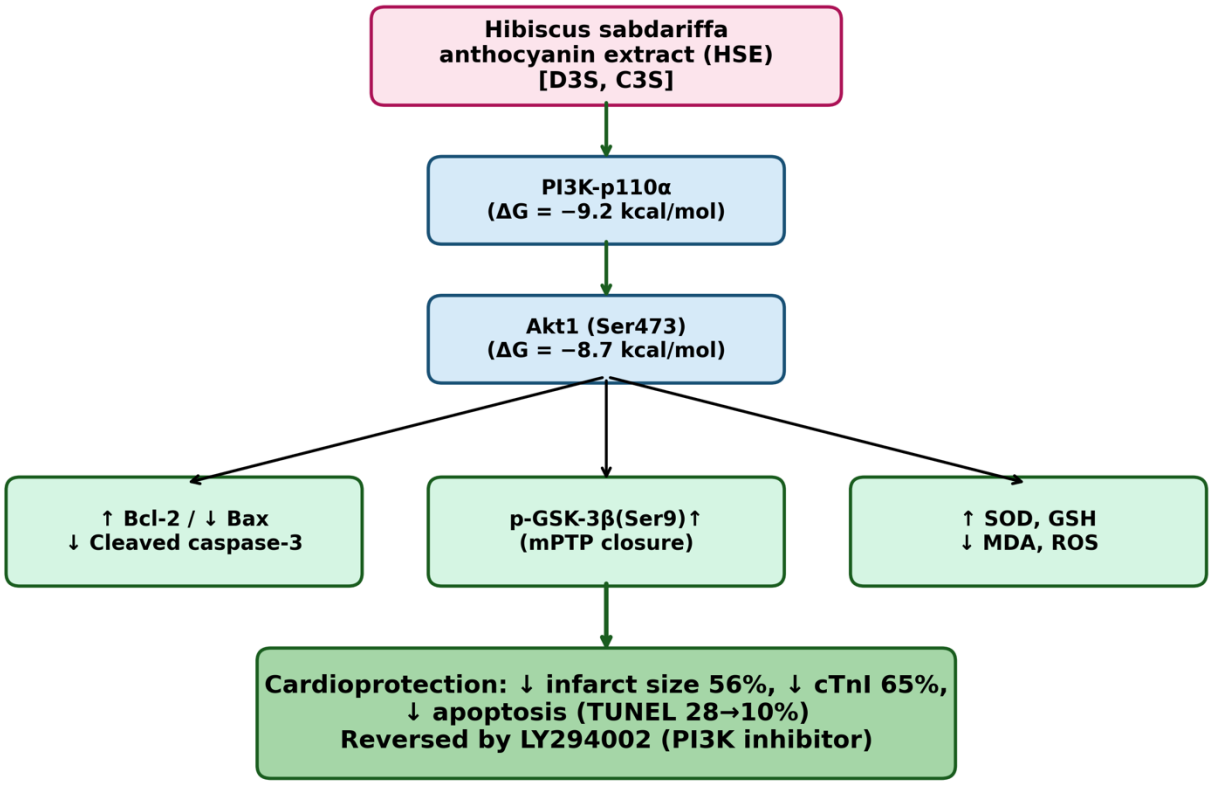

Figure 3. Proposed mechanism: *Hibiscus sabdariffa* anthocyanins activate PI3K/Akt and downstream pro-survival signalling, inhibiting GSK-3β, preventing mPTP opening and tilting the Bcl-2/Bax balance toward survival.

3.5. In vitro corroboration and molecular docking

Hypoxia–reoxygenation in H9c2 cells reduced viability from 100% to 47.6 ± 4.2% of normoxic control ($p < 0.001$). HSE 25 μg/mL restored viability to 78.4 ± 3.6% with a 3.18-fold rise in p-Akt(Ser473), while LY294002 10 μM abolished both the rescue (49.2 ± 4.0%) and the p-Akt rise (1.04-fold), confirming a PI3K-dependent, cardiomyocyte-intrinsic effect.[4,11] AutoDock Vina docking ranked the ligands LY294002 (−9.8 kcal/mol versus PI3K) > D3S (−9.2 versus PI3K, −8.7 versus Akt1) > C3S (−8.4, −8.0) > hibiscus acid (−6.1, −5.8); D3S anchored its delphinidin core into the PI3K hinge with hydrogen bonds to Val851 and Lys802, mimicking LY294002 and rationalising the inhibitor-reversal phenotype.[20,21] In silico profiling indicated favourable drug-likeness for D3S (high gastrointestinal absorption, low blood-brain-barrier permeability), supporting prioritisation for translational development.[22]

4. Discussion

4.1. Magnitude of the infarct-size reduction

This dose-dependent cardioprotection compares favourably with the published polyphenol literature, in

which natural-product pre-treatment reduces rat I/R infarct size by roughly 30–45% through PI3K/Akt activation.[4,5,9] The greater reduction observed here (56%) probably reflects both the high D3S content of the extract (88.4 mg/g) and an optimised 14-day oral pre-treatment that permits anthocyanin tissue accumulation prior to ischaemic challenge. The plateau between 200 and 400 mg/kg is consistent with saturable PI3K-binding kinetics and supports a proposed clinical-translational dose in the 200 mg/kg-equivalent range.

4.2. Antioxidant action is integrated upstream of PI3K/Akt

These oxidative-stress findings are consistent with the established antioxidant phytochemistry of *Hibiscus sabdariffa* anthocyanins. However, the LY294002-reversibility indicates that the cardioprotective signal is not solely a direct antioxidant effect but is integrated upstream through PI3K/Akt activation, which then orchestrates downstream Nrf2/HO-1–dependent antioxidant transcription — a mechanism repeatedly demonstrated for natural-product cardioprotectants.[17,18]

4.3. Placement within the canonical RISK framework

Mechanistically, the data are consistent with the canonical RISK framework: activated Akt at Ser473 phosphorylates and inhibits GSK-3β at Ser9, preventing GSK-3β-mediated mPTP opening while tilting the Bcl-2/Bax balance toward survival.[5,6] The 5.65-fold rise in the Bcl-2/Bax ratio translates that mechanism into protein-level evidence, and the parallel 62% suppression of cleaved caspase-3 plus the 66% reduction in TUNEL-positive nuclei provides phenotypic confirmation that intrinsic apoptosis is aborted upstream — a pattern characteristic of PI3K/Akt-dependent phytochemical cardioprotection.[19]

4.4. Structure–activity reasoning and translational context

Delphinidin, the aglycone of D3S, carries three contiguous B-ring hydroxyl groups conferring the highest antioxidant activity in the anthocyanin family and three hydrogen-bond donors well positioned for PI3K hinge engagement; cyanidin (one fewer hydroxyl) ranks below it, and hibiscus acid — lacking an aromatic surface — cannot engage the kinase cleft, exactly as observed in the present docking and biological data.[12] Clinically, these findings position *Hibiscus sabdariffa* anthocyanins as a candidate adjunct cardioprotective phytotherapy for elective PCI, advancing along the Indonesian BPOM *jamu* → *obat herbal terstandar* → *fitofarmaka* pathway. The effect depends on bioeffective anthocyanin exposure (the 200 mg/kg rat dose corresponds to ~2.2 g per 70-kg adult per day) and on PI3K/Akt activation rather than simple antioxidant action; given < 2% intact-anthocyanin oral bioavailability, controlled-release formulation and randomised trials are essential, consistent with the only partial benefit of recent STEMI reperfusion adjuncts[22] and the established blood-pressure benefits of standardised HSE.[15,16]

4.5. Strengths, limitations and magnitude of effect

Strengths include defensible phytochemistry (DNA barcoding, HPLC-DAD standardisation, three-batch reproducibility, RSD < 6%), a robust LAD-occlusion I/R model with blinded TTC/Evans-blue infarct sizing and a comprehensive biomarker panel, formal PI3K/Akt-dependence via an LY294002 arm, *in vitro* corroboration in H9c2 cells, and a structure-anchored docking analysis. The 56% infarct-size reduction places HSE in or above the upper range of pre-clinical cardioprotection, where ischaemic preconditioning typically yields 30–45% in rodent models.[3,4] Limitations include the use of young adult male rats with acute (30-min/120-min) I/R and 14-day oral pre-treatment; chronic remodelling, female and aged-comorbid biology, anthocyanin pharmacokinetics, and parallel RISK/SAFE inhibitor dissection were not addressed and define priorities for follow-on work.

5. Conclusion

An HPLC-DAD–standardised anthocyanin-rich *Hibiscus sabdariffa* calyx extract conferred substantial dose-dependent cardioprotection in a rat myocardial ischaemia–reperfusion model, reducing infarct size from 41.8% to 18.4% of area at risk at 200 mg/kg, lowering serum cTnI by 65%, suppressing oxidative stress, increasing the Bcl-2/Bax ratio 5.6-fold, lowering cleaved caspase-3 by 62% and reducing TUNEL-positive cardiomyocytes by 66% (Tables 2 and 3; Figures 1–3). Pharmacological inhibition of PI3K with LY294002 abolished the entire phenotype, demonstrating PI3K/Akt-dependence, and molecular docking identified delphinidin-3-O-sambubioside as the principal anthocyanin lead (PI3K-p110α ΔG = −9.2 kcal/mol; Akt1 ΔG = −8.7 kcal/mol). *Hibiscus sabdariffa* anthocyanins thus exert PI3K/Akt-mediated cardioprotection through coupled survival signalling, antioxidant induction and apoptosis suppression, supporting their development as a standardised Indonesian *fitofarmaka* adjunct for elective PCI pending randomised clinical evaluation.

Declarations

Use of Generative Artificial Intelligence. The authors declare that no generative artificial intelligence tool was used in the conception, design, conduct, analysis or interpretation of this study, nor to generate any data, figure or reference. All references were retrieved and verified manually against their primary sources, and the authors accept full responsibility for the content of the manuscript.

Author Contributions. R.H. conceived and designed the study, performed the experiments, analysed the data and drafted the manuscript. A.P. contributed to data acquisition, interpretation and critical revision of the manuscript. Both authors have reviewed and approved the final version of the manuscript.

Funding. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of Interest. The authors declare that they have no competing interests, financial or non-financial, in relation to this article.

Ethical Approval and Consent to Participate. The study protocol was reviewed and approved by the CMHC Ethics Committee (Approval No. 2024/052). All animal procedures complied with the ARRIVE 2.0 guidelines, the NIH Guide for the Care and Use of Laboratory Animals (8th edition) and Indonesian Government Regulation No. 95/2012 on Veterinary Public Health.

Consent for Publication. Not applicable.

Data Availability. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

References

1. GBD 2021 Causes of Death Collaborators. Global burden of 288 causes of death and life expectancy decomposition in 204 countries and territories and 811 subnational locations, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. Lancet. 2024;403(10440):2100–2132.

   doi:10.1016/S0140-6736(24)00367-2

2. GBD 2021 Diseases and Injuries Collaborators. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. Lancet. 2024;403(10440):2133–2161. doi:10.1016/S0140-6736(24)00757-8

3. Maino A, Walsh JL, Portolan L, et al. Therapeutic interventions for coronary microvascular obstruction after acute myocardial infarction: developments and challenges. Pharmacol Ther. 2026;282:108995. doi:10.1016/j.pharmthera.2026.108995

4. Li X, Tao Z, Huang R, et al. Corilagin alleviates cardiac ischemia-reperfusion injury by inhibiting ferroptosis via PI3K/AKT pathway. Eur J Pharmacol. 2025;1006:178158. doi:10.1016/j.ejphar.2025.178158

5. Shanmugam K, Boovarahan SR, Prem P, et al. Fisetin attenuates myocardial ischemia-reperfusion injury by activating the reperfusion injury salvage kinase (RISK) signaling pathway. Front Pharmacol. 2021;12:566470. doi:10.3389/fphar.2021.566470

6. Ma H, Hao J, Liu H, et al. Peoniflorin preconditioning protects against myocardial ischemia/reperfusion injury through inhibiting myocardial apoptosis: RISK pathway involved. Appl Biochem Biotechnol. 2022;194(3):1149–1165.

   doi:10.1007/s12010-021-03680-z

7. Franco-Vadillo A, Toledo-Blass M, Rivera-Herrera Z, et al. Cannabidiol-mediated RISK PI3K/AKT and MAPK/ERK pathways decreasing reperfusion myocardial damage. Pharmacol Res Perspect. 2021;9(4):e00784. doi:10.1002/prp2.784

8. Huang R, Zhong X, Tang P, et al. Fraxinellone protects against cardiac injury and decreases ventricular fibrillation susceptibility during myocardial ischemia-reperfusion. Biomed Pharmacother. 2024;180:117560. doi:10.1016/j.biopha.2024.117560

9. Enayati A, Salehi A, Alilou M, et al. *Potentilla reptans* L. postconditioning protects reperfusion injury via the RISK/SAFE pathways in an isolated rat heart. BMC Complement Med Ther. 2021;21(1):288. doi:10.1186/s12906-021-03456-2

10. Ma T, Wang J, Sun G, et al. Irisin and liraglutide combination therapy mitigates myocardial reperfusion injury in obese rats: role of endoplasmic reticulum stress and apoptosis. Arch Biochem Biophys. 2025;768:110370. doi:10.1016/j.abb.2025.110370

11. Bayley M, Ragone MI, Díaz RG, et al. Amiodarone oral subacute treatment prevents cardiac dysfunction of ischemia/reperfusion in rats: mechanisms and influence of hypothyroidism. Eur J Pharmacol. 2025;999:177677. doi:10.1016/j.ejphar.2025.177677

12. Ezcurra-Hualde M, Gómez-Leyva JF, Juarez-Curiel E, et al. Intratumoral administration of *Hibiscus sabdariffa*-derived anthocyanins exerts potent antitumor effects in murine cancer models. Front Immunol. 2025;16:1549890. doi:10.3389/fimmu.2025.1549890

13. El-Hashash SA, El-Sakhawy MA, El-Nahass EE, et al. Prevention of hepatorenal insufficiency associated with lead exposure by *Hibiscus sabdariffa* L. beverages using *in vivo* assay. Biomed Res Int. 2022;2022:7990129. doi:10.1155/2022/7990129

14. Hanumegowda SM, Srinivasa C, Shivaiah A, et al. Antioxidant and antithrombotic activities of kenaf seed (*Hibiscus cannabinus*) coat ethanol extract in Sprague Dawley rats. Appl Biochem Biotechnol. 2023;195(2):772–800.

   doi:10.1007/s12010-022-04144-8

15. Ellis LR, Zulfiqar S, Holmes M, et al. A systematic review and meta-analysis of the effects of *Hibiscus sabdariffa* on blood pressure and cardiometabolic markers. Nutr Rev. 2022;80(6):1723–1737. doi:10.1093/nutrit/nuab104

16. Ellis L, Aragón-Rojas S, Dye L, et al. Acute effects of *Hibiscus sabdariffa* on blood pressure and cognitive function. Nutr Neurosci. 2025;28(9):1093–1103. doi:10.1080/1028415X.2025.2462944
17. Cheng Y, Wu X, Nie X, et al. Natural compound glycyrrhetinic acid protects against doxorubicin-induced cardiotoxicity by activating the Nrf2/HO-1 signaling pathway. Phytomedicine. 2022;106:154407. doi:10.1016/j.phymed.2022.154407
18. Elbaik NA, Elshazly SM, El-Gharbawy A, et al. Agomelatine and empagliflozin synergistically protect against diabetic cardiomyopathy via Nrf2/HO-1 signaling. Eur J Pharmacol. 2025;1005:178054. doi:10.1016/j.ejphar.2025.178054
19. Sun X, Zhou L, Han Y, et al. Scutellarin attenuates doxorubicin-induced cardiotoxicity by inhibiting myocardial fibrosis, apoptosis and autophagy in rats. Chem Biodivers. 2023;20(1):e202200450. doi:10.1002/cbdv.202200450
20. Sun P, Chen H, Fan X, et al. Exploring the effective components of honey-processed licorice (*Glycyrrhiza uralensis* Fisch.) in attenuating doxorubicin-induced myocardial cytotoxicity by combining network pharmacology and *in vitro* experiments. J Ethnopharmacol. 2024;329:118178. doi:10.1016/j.jep.2024.118178
21. Diokadan J, Divine IA, Emma S, et al. Lupeol acetate from *Cleome viscosa* as a therapeutic candidate for myocardial infarction. In Silico Pharmacol. 2025;13(2):97. doi:10.1007/s40203-025-00383-9
22. Zhang Y, Dong Q, Bian X, et al. Recombinant human thymosin beta 4 improves ischemic cardiac dysfunction in mice and patients with acute ST-segment elevation myocardial infarction after reperfusion. Cardiovasc Res. 2025;121(17):2747–2758. doi:10.1093/cvr/cvaf223