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MELATONIN-MEDIATED CARDIOPROTECTION ACROSS EXPERIMENTAL MODELS OF CARDIAC INJURY: A CRITICAL NARRATIVE REVIEW WITH PRIMARY-SOURCE QUANTITATIVE COMPARISONS

By

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Abstract

Background: Experimental studies have reported cardioprotective effects of melatonin across ischemic, toxic, metabolic, inflammatory and hemodynamic forms of cardiac injury, but neither the magnitude nor the biological basis of protection is likely to be uniform across etiologies. Methods: We conducted a structured critical narrative review centered on primary-source verification. Candidate studies were identified through targeted, iterative literature searches and citation tracking rather than through a prospectively registered exhaustive search workflow. Controlled in vivo mammalian comparisons of melatonin versus an appropriate injury control formed the quantitative core, while in vitro experiments were used for mechanistic interpretation. Numerical contrasts were retained only after verification of group identity, sample size, central estimate, dispersion, unit or scale, and time point in the primary source. Ratio of means (RoM) with 95% confidence intervals was used as a descriptive study-level effect measure for positive continuous outcomes when biologically appropriate. Repeated measurements, shared controls and potentially overlapping cohorts were not treated as independent. Results: Primary-source-verified comparisons showed a broadly protective direction but substantial between-model variation. In anthracycline injury, corrected data from Pehlivan et al. showed lower CK-MB (RoM 0.543, 95% CI 0.408–0.722), MDA (0.564, 0.364–0.872) and IL-6 (0.301, 0.209–0.432) with melatonin. Othman et al. showed lower CK-MB (0.458, 0.430–0.488) and cardiac TBARS (0.618, 0.590–0.647) with higher cardiac GSH (2.265, 2.167–2.367), while Liu et al. (2018) provided a quantitative reduction in myocardial apoptosis (0.354, 0.273–0.459) together with perturbation-supported AMPK/PGC-1α evidence. In myocardial ischemia/reperfusion, infarct size was lower with melatonin (0.674, 0.609–0.746); isoproterenol injury showed lower cTnT (0.504, 0.441–0.576); and endotoxemia showed lower CK-MB (0.547, 0.437–0.685) and TBARS (0.474, 0.384–0.586). In diabetic rats with recovered individual-level supplementary data, 4-HNE, troponin-T, CK-MB and LDH were lower, whereas GSH and GPx were higher. Raw cardiopulmonary bypass data similarly showed lower CK-MB, MDA and IL-6 and higher SOD at T2, while echocardiographic effects remained imprecise. A trastuzumab model broadened the cancer-therapy evidence but remained qualitative because exact endpoint-level group statistics were not tabulated. Perturbation experiments supported model-specific roles for PER2, AMPK/PGC-1α, YAP/ACSL4, SIRT1/Nrf2, endothelial LRRC8A–AKT/eNOS/HIF-1α and MT2/SIRT1/Nrf2. A small randomized human trial was considered separately as translational context and was not combined with animal comparisons. Conclusions: Melatonin-associated cardioprotection is directionally reproducible across several experimental etiologies, but the evidence does not support a single universal effect size or mechanism. The most defensible interpretation is a model-dependent protective phenotype involving redox control, mitochondrial preservation, inflammatory modulation and regulated

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cell death pathways, with timing, dose, route and cardiac compartment as plausible effect modifiers.

Keywords: *melatonin; cardioprotection; doxorubicin; ischemia/reperfusion; oxidative stress; mitochondria; inflammation; narrative review; primary-source verification; ratio of means*

1. Introduction

Melatonin is an endogenous indoleamine best known for circadian signaling, but its cardiovascular actions also include direct and indirect antioxidant effects, modulation of inflammatory signaling, and effects on mitochondrial homeostasis. Experimental cardioprotection has been reported in myocardial ischemia/reperfusion (I/R), infarction, catecholamine injury, cancer therapy-associated cardiotoxicity, including anthracycline and trastuzumab injury, diabetes, sepsis/endotoxemia, pulmonary vascular disease, cardiopulmonary bypass and post-resuscitation myocardial dysfunction. These models differ substantially in initiating insult, ventricular compartment, treatment schedule and the biological meaning of their outcome measures; consequently, evidence of benefit across models does not imply that the same mechanism or effect size applies to all of them (Mao *et al*., 2020; Luo *et al*., 2025; Prestes *et al*., 2026).

The mechanistic literature has also moved beyond simple expression associations. In time-of-day I/R experiments, PER2 knockdown attenuated melatonin-associated protection, supporting a chronobiological PER2-dependent component (Luo *et al*., 2025). In acute doxorubicin injury, pharmacological and siRNA experiments implicated AMPK/PGC-1α signaling (Liu *et al*., 2018). In separate doxorubicin models, YAP knockdown and verteporfin diminished protection against oxidative injury and ferroptosis, and inhibition of SIRT1 or Nrf2 reversed protection against oxidative stress, pyroptosis and apoptosis (Li *et al*., 2020; Sun *et al*., 2022; Zhang *et al*., 2023). Endothelial LRRC8A deletion or blockade eliminated the functional and angiogenic benefit of melatonin after myocardial infarction, whereas Nrf2 ablation and pharmacological interference with MT2/SIRT1/Nrf2 substantially reduced protection in diabetic cardiomyopathy (Jie *et al*., 2026; Liu *et al*., 2026). These perturbation experiments warrant stronger causal language than studies in which a pathway marker merely changes in parallel with cardiac protection.

Cross-study quantitative comparison is challenging because the literature spans distinct biological outcomes, units, matrices and repeated time points from the same animals. Historical anthracycline studies also illustrate the risk of relying on secondary extraction tables when original treatment arms are not transparently mapped. This review therefore emphasizes primary-source traceability and descriptive study-level comparisons rather than a formal pooled synthesis.

The objective was to determine whether melatonin produces a reproducible cardioprotective phenotype across experimental etiologies while preserving distinctions among functional, biochemical, redox, inflammatory, mitochondrial and regulated cell death outcomes. A second objective was to identify plausible effect modifiers and to grade mechanistic inferences according to whether the proposed pathway had been experimentally perturbed.

2. Methods

2.1 Review approach and evidence selection

This article was developed as a structured critical narrative review with primary-source quantitative comparisons. Candidate primary studies were identified iteratively through targeted searches of the biomedical literature, backward and forward citation tracking, and retrieval of full primary reports and supplementary material when available. No prospectively registered protocol or complete exhaustive search-and-screening log was maintained; accordingly, the article is not presented as a systematic review and does not claim exhaustive capture of all relevant studies. Controlled *in vivo* mammalian experiments were prioritized for quantitative comparison when melatonin alone was compared with an appropriate injury control. The retrieved evidence base covered myocardial I/R, myocardial infarction, isoproterenol injury, anthracycline/doxorubicin cardiotoxicity, cisplatin cardiotoxicity, trastuzumab-associated cardiotoxicity, diabetic cardiac injury, sepsis/endotoxemia, pulmonary arterial hypertension/right-ventricular injury, post-resuscitation myocardial dysfunction and cardiopulmonary bypass.

In vitro experiments were retained only for mechanistic interpretation and were not combined with animal outcomes. Combination-treatment studies contributed only when a separate melatonin-alone arm was available. Left- and right-ventricular phenotypes were kept distinct. Repeated measurements from the same animals were not treated as independent comparisons; shared controls were not duplicated; and cohorts with a plausible risk of overlap were not counted twice unless independence could be reasonably established from the primary reports. Human clinical evidence was considered only as a separate translational context and was not incorporated into the animal quantitative comparisons.

2.2 Data extraction and primary-source verification

For each comparison selected for quantitative reporting, extraction recorded species/strain when available, sex, group size, injury model, melatonin dose, route, timing, comparator, outcome definition, unit, central estimate, dispersion measure and time point. Numerical information was accepted in the following hierarchy: individual-level or author-provided supplementary data; values printed in the primary article; and controlled graph digitization only as a last resort. A value was not retained for quantitative comparison if group identity, sample size, unit, dispersion or cohort independence remained uncertain.

Data were classified as (1) directly reported in the primary article, (2) derived from primary data by a transparent calculation or conversion, or (3) extracted from a graph.

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When individual observations were available in author-supplied supplementary files, arithmetic means and sample SDs were recomputed directly from those observations and the published mean ± standard error of the mean (SEM) summaries were used as a cross-check rather than as the calculation source. SEM was converted to standard deviation only when the original paper explicitly identified the reported dispersion as SEM, using SD = SEM × √n.

If a paper did not label its dispersion, the endpoint remained excluded unless another statistic in the same primary report resolved the ambiguity. In Zhang *et al*. (2013), n=8/group and the published t statistics reproduce the tabulated ± values only when those values are treated as SD; this was therefore recorded as a derived/validated dispersion assignment rather than as directly reported SD. In the cardiopulmonary bypass study, T2 (120 min after operation) was selected as the primary repeated-measures time point to avoid treating serial measurements from the same animals as independent.

2.3 Study-level quantitative comparisons

For strictly positive continuous outcomes reported on biologically interpretable scales, the principal study-level effect ratio was the ratio of means (RoM), defined as the melatonin-treated injury mean divided by the injury-control mean. Confidence intervals were calculated on the log scale and exponentiated. RoM values below 1 therefore indicate lower values with melatonin; whether this is favorable depends on the biological direction of the outcome. For outcomes in which higher values are favorable, such as antioxidant activity or contractile performance, RoM values above 1 indicate a favorable shift. Hedges g was not used merely to force biologically distinct outcomes onto a common scale.

No pooled estimate of cardioprotection was calculated. The verified comparisons represented different etiologies, biological outcomes, assay matrices and experimental schedules, and several domains contained only one or two independent experiments. RoMs and their 95% confidence intervals were therefore used descriptively to summarize study-level contrasts, not to estimate a common treatment effect across models. Interpretation remained anchored to the biological direction and design of each experiment and to the structured narrative review.

2.4 Mechanistic evidence grading

Mechanistic claims were graded in three tiers: Level 1, perturbation-supported evidence, in which genetic knockdown/knockout or pharmacological blockade diminished melatonin-associated protection; Level 2, intervention-associated evidence, in which melatonin changed the pathway together with the cardiac phenotype without a dependency experiment; and Level 3, descriptive association. Terms such as “required for”, “dependent on” or “mediates” were reserved for Level 1 evidence when the experiment justified that wording.

3. Evidence synthesis

3.1 Evidence landscape and study characteristics

The primary-source-verified evidence base spanned ischemic, toxic, metabolic, inflammatory, pulmonary vascular, extracorporeal circulation and resuscitation-associated injury models. The study-level characteristics, injury models, melatonin exposures and evidence contribution of each retained experiment are summarized in Table 1.

Table 1. Experimental studies retained in the structured evidence review.

Study	Model	Animals / design	Melatonin exposure	Evidence included in synthesis
Xu *et al*., 2001	Doxorubicin cardiotoxicity	Male Sprague-Dawley rats; source Figure 2: n=7 DOX, n=9 DOX+MLT	6 mg/kg/day i.p.; 15 d; DOX 3 mg/kg i.v. ×4 (12 mg/kg cumulative)	Time-to-peak dV/dt - Quantitative; remaining hemodynamic and lipid-peroxidation outcomes graph-reported
Agapito *et al*., 2001	Adriamycin cardiotoxicity	Male Wistar rats; n=6/group	50 µg/kg/day i.p. for 10 d; single ADR 20 mg/kg i.p.	Cardiac GSSG/GSH - Quantitative from primary tables; lipid peroxidation and GPx graph-reported
Liu *et al*., 2002	Doxorubicin cardiotoxicity	Rat model; n=6/group for hemodynamics	Melatonin vs DOX	LVEDP, LVESP, ±dP/dt, stroke volume, cardiac output - Quantitative
Acikel *et al*., 2003	Isoproterenol injury	Rats; n=8/group	Melatonin vs ISO	cTnT, cTnI, histology - Quantitative
Sahna *et al*., 2003	Doxorubicin cardiotoxicity	Intact and pinealectomized rats; n=8/group	4 mg/kg i.p.; before/after DOX	Cardiac MDA and morphology - Quantitative; multiple arms share controls and are not independent
Dziegiel *et al*., 2003	Doxorubicin cardiotoxicity	Buffalo rats; n=10/group	20 mg/kg s.c.; repeated with DOX	GSH, MDA+4-HDA, CAT, SOD, GPx - Qualitative; exact means are graph-only and were not digitized
Balli *et al*.,	Doxorubicin	Male Wistar rats;	6 mg/kg/day i.p.; 15 d	Cardiac MDA - Quantitative

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2004	cardiotoxicity	n=8/group		
Öz *et al*., 2006	Doxorubicin cardiotoxicity	Male Wistar rats; six groups; n=10/group	10 mg/kg s.c.; sequence-specific exposure	MDA, GSH, CPK-MB, ultrastructure - Quantitative; DM/MD share the DX control; sequence-dependent phenotype
Othman *et al*., 2008	Adriamycin cardiotoxicity	Male Sprague-Dawley rats; 10/group allocated; biochemical tables n=8	15 mg/kg/day; 5 d before + 5 d after ADR	CK-MB, LDH, cardiac TBARS/GSH and related redox markers - Quantitative from primary tables
Zhang *et al*., 2013	Adriamycin cardiotoxicity	Rat breast-cancer model; n=8/group for tissue analysis	Melatonin + Adriamycin	LPO, SOD, GSH-Px - Quantitative; dispersion validated as SD from reported t statistics
Bilginoğlu *et al*., 2014	Adriamycin cardiotoxicity	Male Wistar-Albino rats; n=7/group	Melatonin + Adriamycin	CK-MB, LDH, AST, cTnT - Quantitative; original dispersion SEM
Liu *et al*., 2018	Acute doxorubicin cardiotoxicity	C57BL/6 mice; n=20/group allocated; n=6 for key quantified figures + H9c2	20 mg/kg/day i.p.; DOX 20 mg/kg cumulative	*In vivo* apoptosis - Quantitative; mitochondria and AMPK/PGC-1α - Mechanistic Level 1 (siRNA + Compound C)
Aygun & Gul, 2019	Doxorubicin cardiotoxicity	Rat model	Melatonin arm separate from agomelatine	cTnT, CK; ECG/scintigraphic endpoints - Quantitative
Pehlivan *et al*., 2020	Doxorubicin cardiotoxicity	Male Wistar rats; 32 allocated; final DXR n=6, DXR+MEL n=7	10 mg/kg/day i.p.; 7 d; DOX 45 mg/kg i.v. day 5	CK-MB, MDA, IL-1/IL-6/IL-18, NO and histology - Quantitative; corrected primary Table 4 extraction
Durdagi *et al*., 2021	Doxorubicin cardiotoxicity	Male Wistar rats; 32 total; final control n=6, DOX n=8, DOX+MEL n=6, DOX+ADM n=7	10 mg/kg/day i.p.; 7 d; DOX 45 mg/kg i.v. day 5	ECG, histology, inflammatory/redox/injury markers - Distinct qualitative study; graph-only biochemical means not digitized
Yang *et al*., 2020	Doxorubicin injury	H9c2 cardiomyoblasts	Melatonin + DOX	AMPKα2 mitochondrial translocation/function - Mechanistic; *in vitro* only
Li *et al*., 2020	Doxorubicin cardiotoxicity	H9c2 + mouse model	Melatonin + DOX	YAP, ROS, apoptosis, mitochondrial membrane potential - Mechanistic Level 1 (YAP siRNA)
Huang *et al*., 2021	Cardiopulmonary bypass	Male Sprague-Dawley rats; n=6/group	20 mg/kg/day i.p.; 7 d pre-CPB	T2 echo; IL-6, IL-1β, SOD, GSH-Px, MDA, CK-MB, cTnT; ROS/apoptosis/autophagy - Quantitative from raw supplement; AKT/STAT3/mTOR signaling intervention-associated
Abdulwahab *et al*., 2021	T2DM cardiac injury	Male Wistar rats; n=5/group	10 mg/kg gavage; 15 d	Redox, injury biomarkers, cytokines, apoptosis IHC - Quantitative from individual supplementary data
Arinno *et*	Doxorubicin	Male Wistar rats;	MEL 10 mg/kg/day	LV function/hemodynamics;

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al., 2021	cardiotoxicity	32 total; control n=8; DOX n=24 subdivided n=8/group	oral gavage for 30 d; DOX 3 mg/kg i.p. ×6 (18 mg/kg cumulative)	cTnI/NT-proBNP; MDA/inflammation; mitochondrial respiration, ROS, membrane potential, dynamics, biogenesis/bioenergetics; autophagy; apoptosis - Full primary article verified; intervention-associated; 2023 corrigendum mapped to representative p62 and pro-caspase-3 blots; no RoM because endpoint-specific n and exact means are not numerically tabulated
Sun *et al*., 2022	Doxorubicin cardiotoxicity	H9c2, zebrafish, SD rats	Melatonin + DOX	YAP/ACSL4, GPX4, ferroptosis - Mechanistic Level 1
Zhuo & Jiang, 2022	Cisplatin cardiotoxicity	Wistar rats; six groups	5 or 10 mg/kg; 15 d	cTnT, LDH-1, BDNF, TNF-α, GSH, apoptosis - Qualitative/mechanistic
Arinno *et al*., 2023	Trastuzumab cardiotoxicity	Male Wistar rats; 32 total; control n=8; Trz n=24 subdivided n=8/group	10 mg/kg/day oral for 7 d; Trz 4 mg/kg/day i.p. for 7 d	LVEF/LVFS, cTnI, NT-proBNP, inflammation, MDA/GPx4, mitochondrial function/dynamics, autophagy, apoptosis - Qualitative/mechanistic; intervention-associated; exact group statistics not numerically tabulated
Di *et al*., 2023	CLP sepsis	Mice; n=6 for key assays	Pretreatment/post-treatment arms	SV, CO, LV volumes, redox, ER stress, mitochondria, AMPK - Mechanistic Level 1 for AMPK contribution
Zhang *et al*., 2023	Doxorubicin cardiotoxicity	H9c2 + C57BL/6 mice	Melatonin + DOX	SIRT1/Nrf2, oxidative stress, pyroptosis, apoptosis - Mechanistic Level 1
Lazarević *et al*., 2024	LPS endotoxemia	Rats; n=6/group	50 mg/kg oral gavage	CK-MB, LDH, TBARS, inflammation/apoptosis - Quantitative
Luo *et al*., 2025	Myocardial I/R	Mice; n=6 for key endpoints + H9c2	ZT1 vs ZT13 pretreatment	Infarct/function/apoptosis, PER2 - Mechanistic Level 1; timing modifier
Wang *et al*., 2025	Myocardial I/R	Rat hearts and mice; n=6/group	20 mg/kg in mouse protocol	Infarct size, CK-MB, SOD, ferroptosis markers - Quantitative
Jie *et al*., 2026	Myocardial infarction	Mouse MI + endothelial LRRC8A knockout	Melatonin	Systolic function, angiogenesis, LRRC8A–AKT/eNOS/HIF-1α - Mechanistic Level 1
Liu *et al*., 2026	Diabetic cardiomyopathy	STZ mice + primary cardiomyocytes	Melatonin	Function, senescence, MT2/SIRT1/Nrf2 - Mechanistic Level 1
Shi *et al*., 2026	Post-resuscitation dysfunction	Rat CA/CPR	10 mg/kg IV, 30 min post-ROSC	Hemodynamics, LV function, mitochondrial dynamics - Qualitative/mechanistic
Prestes *et al*., 2026	ISO injury and MCT-PAH	Two *in vivo* models + H9c2	Melatonin	LV/RV function; NLRP3 in ISO; xanthine oxidase in PAH - Mechanistic Level 2 for NLRP3/XO

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Abbreviations: ADR, Adriamycin; CA/CPR, cardiac arrest/cardiopulmonary resuscitation; CLP, cecal ligation and puncture; CPB, cardiopulmonary bypass; DOX/DXR, doxorubicin; I/R, ischemia/reperfusion; ISO, isoproterenol; MCT-PAH, monocrotaline-induced pulmonary arterial hypertension; T2DM, type 2 diabetes mellitus; MEL/MLT, melatonin. Note: "Quantitative" indicates that at least one outcome had primary-source-verifiable group statistics; "qualitative/mechanistic" indicates that design, direction of effect or mechanistic findings were retained without assigning uncertain group-level numerical estimates.

Full-text recovery of historical anthracycline studies expanded the quantitative evidence beyond the previously verified Sahna, Öz, Balli, Bilginoğlu and Pehlivan datasets: Agapito and Othman now contribute directly tabulated redox or injury-marker outcomes, Xu contributes one directly stated hemodynamic endpoint, and Liu *et al*. (2018) contributes a directly reported *in vivo* apoptosis estimate in addition to its perturbation-supported mechanism. Durdagi *et al*. (2021) is retained as a separate experimental report after direct full-text comparison with Pehlivan *et al*. (2020), although its graph-only biochemical values were not digitized. A distinct trastuzumab cardiotoxicity study contributed multi-domain functional, injury-marker, inflammatory, redox, mitochondrial, autophagy and apoptosis evidence but remained qualitative because exact group-level numerical values were not tabulated in the primary report.

The distribution of evidence remained uneven across etiologies: anthracycline cardiotoxicity contained the densest combination of quantitative and mechanistic information, whereas several other models were represented primarily by functional or pathway-specific findings. Primary-table recovery now supports quantitative anthracycline evidence not only for function, injury biomarkers and redox status, but also for inflammatory and regulated cell death outcomes. The trastuzumab study broadened this landscape beyond classical cytotoxic chemotherapy, with reported findings across functional, injury-biomarker, redox, inflammatory, regulated cell death and mitochondrial domains but no pathway-dependency experiment. To make this heterogeneity explicit without converting category labels into study counts, the evidence domains retained for each injury model are mapped in Figure 1.

Figure 1. Primary-source evidence landscape across experimental cardiac-injury models.

	Function/ structure	Injury biomarkers	Redox/ Oxidative stress	Inflammation	Regulated cell death	Mitochondrial phenotype	Perturbation-supported pathway
Myocardial I/R	reported	quantitative	quantitative	reported	reported		perturbed
Myocardial infarction	reported						Perturbed
Anthracycline/DOX	quantitative	quantitative	quantitative	quantitative	quantitative	reported	perturbed
Trastuzumab injury	reported	reported	reported	reported	reported	reported	
Cisplatin injury		reported	reported	reported	reported		reported
Diabetic cardiomyopathy	reported	quantitative	quantitative	quantitative	quantitative		perturbed
Sepsis/endotoxemia	reported	quantitative	quantitative	reported	reported	reported	perturbed
Isoproterenol injury	reported	quantitative		reported			reported
PAH/RV injury	reported		reported				reported
Cardiopulmonary bypass	quantitative	quantitative	quantitative	quantitative	quantitative		
Post-resuscitation dysfunction	reported	reported				reported	reported

Cells indicate evidence type; categories are not study counts. "Perturbed" denotes pathway-dependency evidence.

Reported/associated | Primary-source quantitative | Perturbation-supported

Note: Cells describe the type of evidence retained in the current synthesis and are not study counts. "Quantitative" denotes an outcome with primary-source-verifiable group statistics; "perturbed" denotes pathway-dependency evidence in which genetic or pharmacological interference attenuated melatonin-associated protection. Full-text recovery of Agapito, Othman, Pehlivan and Liu *et al*. (2018) expands the anthracycline row to quantitative inflammatory and regulated cell death evidence, while graph-only outcomes remain qualitative unless exact group statistics are recoverable.

3.2 Study-level quantitative cardioprotection

Across the primary-source-verified comparisons, melatonin shifted most injury and redox outcomes in a protective direction, but the magnitude varied widely by model and endpoint. Recovery of the full Sahna *et al*. (2003) table expanded the historical anthracycline stratum. In intact rats, myocardial MDA was 57.6 ± 1.7 nmol/g tissue after doxorubicin and 42.8 ± 0.5 when melatonin was started 1 h before doxorubicin (RoM 0.743, 95% CI 0.698–0.791); when melatonin was started 24 h after doxorubicin, MDA was 43.2 ± 2.1 (0.750, 0.671–0.838). In pinealectomized rats, MDA

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was 65.4 ± 0.5 after doxorubicin and 43.9 ± 0.5 with exogenous melatonin (0.671, 0.653–0.690). Agapito *et al*. (2001) provided another directly tabulated cardiac redox contrast (n=6/group; mean ± SEM): cardiac GSSG was 0.34 ± 0.10 µmol/g after Adriamycin and 0.080 ± 0.025 with melatonin (RoM 0.235, 0.101–0.546). Cardiac GSH rose from 0.23 ± 0.12 to 0.55 ± 0.02 µmol/g; the point RoM was 2.391, but its ratio-based CI was wide (0.858–6.665), so the GSSG contrast is the more precise quantitative summary from this study.

Othman *et al*. (2008) further strengthened the historical anthracycline evidence with directly tabulated mean ± SD data (n=8 for the biochemical tables). Relative to Adriamycin alone, melatonin lowered plasma CK-MB from 25.3 ± 1.2 to 11.6 ± 0.9 U/L (RoM 0.458, 95% CI 0.430–0.488), LDH from 380 ± 15 to 281 ± 13 U/L (0.739, 0.709–0.771), and cardiac TBARS from 340 ± 12 to 210 ± 11.7 nmol/g (0.618, 0.590–0.647), while cardiac GSH increased from 2.3 ± 0.12 to 5.21 ± 0.19 µmol/g (2.265, 2.167–2.367). Öz *et al*. (2006) also provided complete primary-source-verifiable data (n=10/group; mean ± SEM): myocardial MDA was lower both when melatonin preceded doxorubicin (MD: 0.532, 0.399–0.710) and when doxorubicin preceded melatonin (DM: 0.436, 0.346–0.550); CPK-MB followed the same sequence-sensitive pattern (MD: 0.646, 0.511–0.817; DM: 0.342, 0.274–0.427), and GSH was higher, particularly in DM (1.874, 1.498–2.344). Zhang *et al*. (2013) provided lower LPO (0.687, 0.612–0.772) with higher SOD (1.962, 1.802–2.135) and GSH-Px (1.540, 1.410–1.681). These historical effects are retained study by study rather than collapsed into a single pooled anthracycline estimate.

The corrected primary-table extraction of Pehlivan *et al*. (2020) materially changed its quantitative contribution. Table 4 shows DXR n=6 and DXR+MEL n=7, with CK-MB 12.40 ± 2.57 versus 6.73 ± 2.11 ng/mg protein (RoM 0.543, 95% CI 0.408–0.722) and MDA 315.57 ± 63.57 versus 177.85 ± 97.45 nmol/mg protein (0.564, 0.364–0.872). The same table provides a strong inflammatory signal: the RoMs were 0.348 (95% CI 0.261–0.465) for IL-1, 0.301 (0.209–0.432) for IL-6, and 0.271 (0.217–0.340) for IL-18; the TNF-α estimate was less precise (0.786, 0.595–1.038).

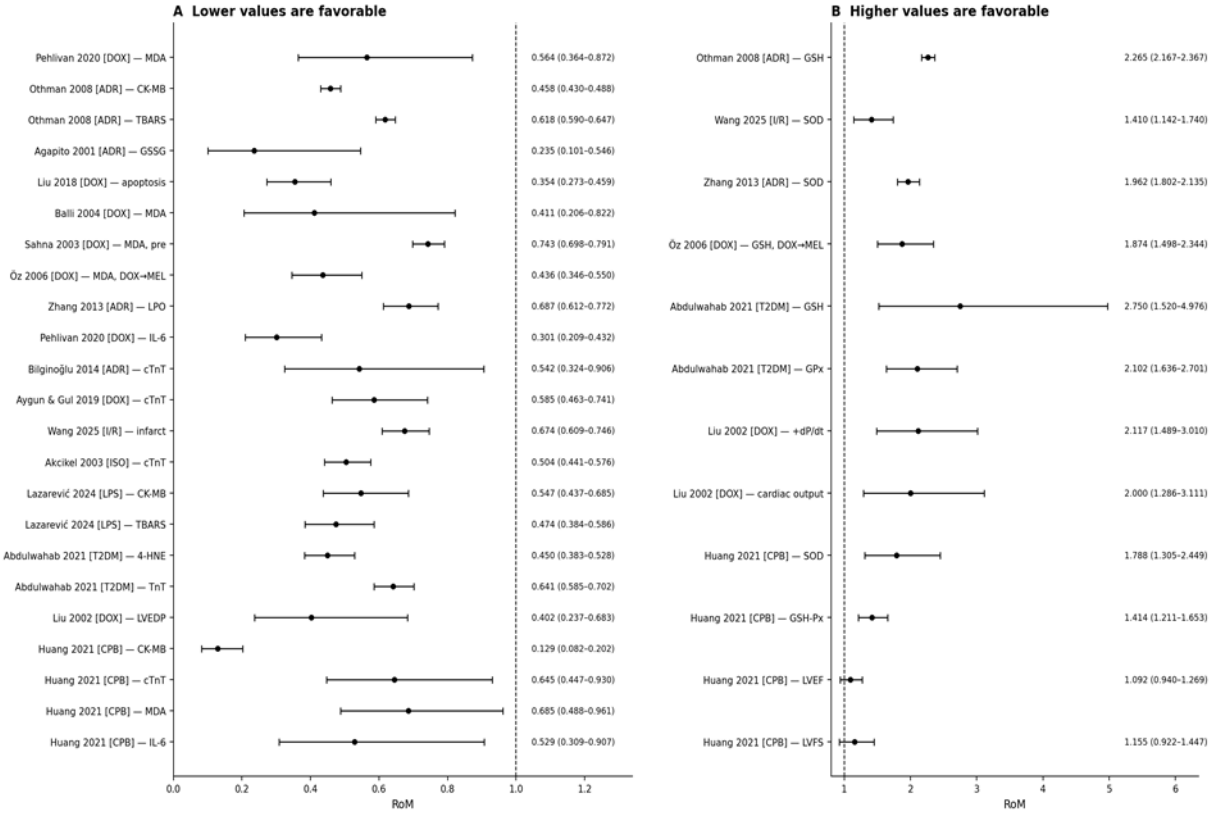
Re-examination of primary Table 4 resolved the apparent discrepancy in SOD values: the DXR+MEL value was 5.87 ± 1.70 *versus* 6.93 ± 1.41 U/mg protein in the DXR group; therefore, this endpoint was not interpreted as evidence of antioxidant restoration. Bilginoğlu *et al*. (2014) reported lower CK-MB (0.660, 0.445–0.977), LDH (0.617, 0.477–0.796), AST (0.645, 0.507–0.820) and cTnT (0.542, 0.324–0.906), while Aygun and Gul (2019) reported lower cTnT (0.585, 0.463–0.741) and CK (0.389, 0.265–0.571).

Direct functional and cell-death data added complementary quantitative information. Liu *et al*. (2002) showed lower LVEDP (RoM 0.402, 0.237–0.683) and higher LVESP (1.614, 1.206–2.160), +dP/dt (2.117, 1.489–3.010), −dP/dt (2.241, 1.433–3.503), stroke volume (1.805, 1.357–2.401) and cardiac output (2.000, 1.286–3.111). Xu *et al*. (2001) directly reported a shorter time to peak dV/dt with melatonin (1.14 ± 0.08 vs 1.33 ± 0.06 ms; mean ± SEM; DOX+MLT n=9, DOX n=7), corresponding to RoM 0.857 (0.728–1.009); its remaining hemodynamic and lipid-peroxidation effects are retained qualitatively because the exact group means are graph-only. In the acute mouse model of Liu *et al*. (2018), myocardial TUNEL positivity fell from 20.68 ± 3.13% to 7.32 ± 2.10% (n=6; mean ± SD), giving RoM 0.354 (0.273–0.459), while AMPK inhibition attenuated the broader protective phenotype. Outside anthracycline injury, Wang *et al*. (2025), Acikel *et al*. (2003), Lazarević *et al*. (2024), Abdulwahab *et al*. (2021) and Huang *et al*. (2021) retained the previously verified quantitative effects. Because multi-arm studies share injury controls and several domains contain biologically heterogeneous endpoints, no pooled estimate was forced. Selected verified study-level effects are summarized in Figure 2.

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Figure 2. Selected study-level quantitative cardioprotection summary.



Note: Ratio of means (RoM) compares melatonin-treated injury with injury control. The plot is separated by biological direction: lower values are favorable for injury markers, oxidized redox products, apoptosis and LVEDP, whereas higher values are favorable for antioxidant or functional outcomes. Pehlivan *et al.* (2020) values are corrected from the primary Table 4. Othman *et al.* (2008) and Agapito *et al.* (2001) use directly tabulated primary values, and Liu *et al.* (2018) apoptosis uses the exact *in vivo* mean ± SD reported in the primary article. For multi-arm Sahna *et al.* (2003) and Öz *et al.* (2006), one representative arm is plotted to avoid visually duplicating a shared injury control; all verified arm-specific estimates are reported in the Results. No pooled estimate is shown.

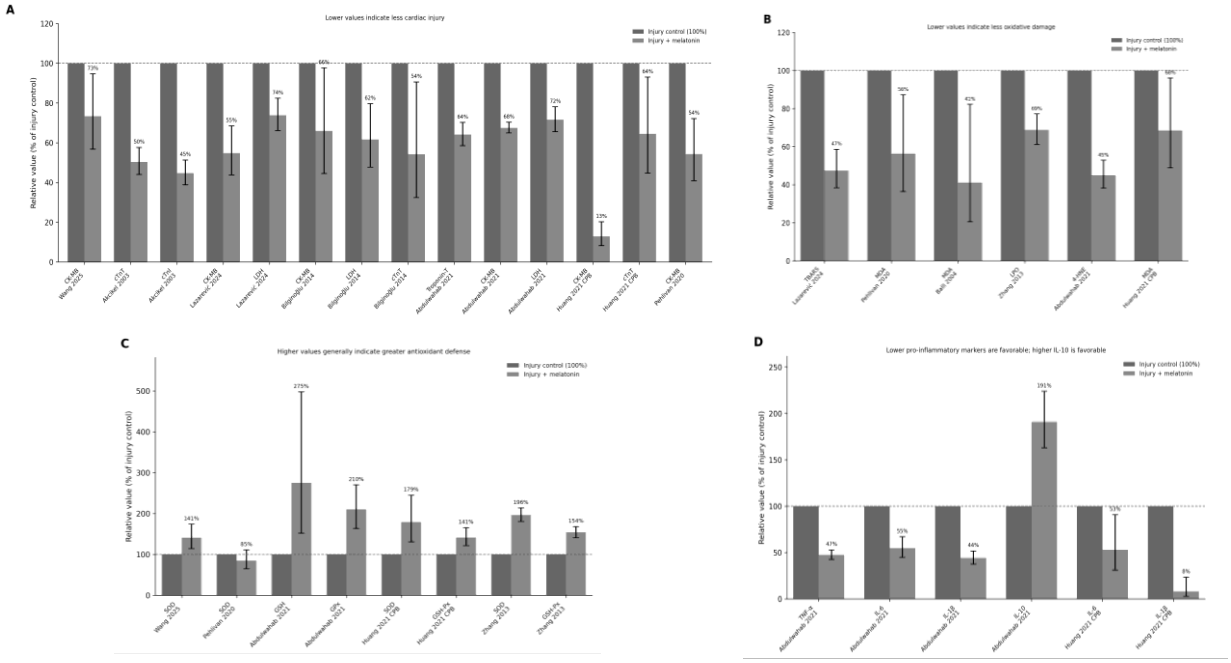
To facilitate cross-domain visual interpretation of the primary-source-verified numerical dataset, the same study-level estimates were rescaled to their matched injury control, which was set to 100%, and organized according to major biological domains in Figure 3. This normalization allows outcomes reported in different units and analytical scales to be displayed within a common visual framework without altering the underlying study-specific effect estimates. The figure therefore provides an intuitive overview of the direction and relative magnitude of melatonin-associated changes across cardiac injury biomarkers, oxidative damage, antioxidant defense, and inflammatory mediators.

Importantly, each bar remains linked to its original experimental comparison and should be interpreted within the context of the corresponding injury model, treatment regimen, biological matrix, and endpoint definition. This representation preserves the study-level structure of the evidence and is intended as a descriptive visualization rather than a statistical aggregation. Accordingly, it does not generate or imply a pooled cross-etiology effect, nor does it assume that differences observed across heterogeneous models are directly interchangeable. Instead, the panel is used to highlight recurring directional patterns while retaining the biological and methodological heterogeneity of the experimental literature.

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Figure 3. Domain-level visualization of primary-source-verified study-level effects of melatonin.



(A) Cardiac injury biomarkers. (B) Oxidative damage. (C) Antioxidant defense. (D) Inflammatory mediators. For each comparison, the matched injury-control mean was normalized to 100%, and the melatonin-treated injury group is expressed relative to that value. The 100% injury-control bar is a normalization reference rather than an error-free measured value; uncertainty is represented by the 95% confidence interval of the underlying RoM for the melatonin contrast. Values below 100% indicate lower levels relative to injury control; for antioxidant defenses and IL-10, values above 100% indicate higher levels. No pooled effect is displayed.

The domain-based view in Figure 3 makes the directional pattern easier to compare without implying a universal cardioprotective effect size. Cardiac injury biomarkers and oxidative-damage measures generally remained below the injury-control reference, whereas antioxidant-defense outcomes were usually higher with melatonin; the Pehlivan *et al*. (2020) SOD result is an exception and is not interpreted as antioxidant restoration. In the inflammatory domain, pro-inflammatory mediators were reduced in the quantitatively resolved diabetic and cardiopulmonary bypass datasets, while IL-10 increased in Abdulwahab *et al*. (2021). The residual spread across studies and endpoints reinforces the decision to retain study-level interpretation rather than force a pooled effect across different etiologies.

3.2 Diabetic cardiac injury: raw-data-derived quantitative stratum

Abdulwahab *et al*. (2021) became quantitatively verifiable after recovery of individual supplementary observations (n=5/group). Recalculation from those observations yielded lower cardiac 4-HNE with melatonin (42.372 ± 3.223 vs 19.052 ± 3.166 SD; RoM 0.450, 95% CI 0.383–0.528), whereas GSH increased from 0.780 ± 0.489 to 2.144 ± 0.547 (RoM 2.750, 1.520–4.976) and GPx from 11.380 ± 2.854 to 23.924 ± 3.291 (2.102, 1.636–2.701).

Serum injury biomarkers were also lower: troponin-T decreased from 111.148 ± 7.568 to 71.228 ± 5.541 pg/mL (RoM 0.641, 0.585–0.702), CK-MB from 323.068 ± 7.347 to 218.192 ± 8.785 U/L (0.675, 0.649–0.703), and LDH from 1034.864 ± 95.530 to 740.444 ± 27.278 U/L (0.716, 0.656–0.781).

The same raw dataset supported a coordinated inflammatory shift. TNF-α (RoM 0.474, 0.425–0.528), IL-6 (0.548, 0.448–0.669) and IL-1β (0.441, 0.376–0.516) were lower, whereas IL-10 was higher (1.910, 1.629–2.239). Apoptosis-related immunohistochemistry showed lower caspase-3 (0.440, 0.378–0.511), p53 (0.467, 0.417–0.523) and Bax (0.453, 0.399–0.513), with higher Bcl-2 (1.770, 1.609–1.947). These markers were retained as a mechanistically coherent phenotype within one experiment and were not treated as independent studies or pooled into a single apoptosis effect.

3.3 Cardiopulmonary bypass and repeated-measures data

Huang *et al*. (2021) randomized 18 male Sprague-Dawley rats to sham, cardiopulmonary bypass (CPB) or melatonin (n=6/group). Melatonin was given intraperitoneally at 20 mg/kg/day for seven days before CPB. The supplemental echocardiographic table reports mean ± SEM at T0, T1 and T2. Because these are repeated observations from the same animals, only T2 (120 min after operation) was used for the study-level contrast to avoid treating repeated measurements as independent.

Recovery of Supplementary Information 6 removed the need for graph digitization of the source article's Figure 2 biomarkers. Using the six individual observations per group at T2, melatonin versus CPB yielded lower CK-MB (RoM 0.129, 95% CI 0.082–0.202), cTnT (0.645, 0.447–0.930),

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MDA (0.685, 0.488–0.961), IL-6 (0.529, 0.309–0.907) and IL-1β (0.080, 0.028–0.233), together with higher SOD (1.788, 1.305–2.449) and GSH-Px (1.414, 1.211–1.653). The raw workbook identifies each observation by endpoint, group and time point but does not restate the source Figure 2 axis units; therefore, the manuscript reports the dimensionless RoM contrasts rather than assigning unverified absolute units. These seven outcomes arise from one experiment and were not treated as independent studies.

At T2, LVEF was 69.94 ± 3.75% in CPB and 76.36 ± 4.18% with melatonin (RoM 1.092, 95% CI 0.940–1.269). LVFS was 40.76 ± 3.03% versus 47.08 ± 4.14%, respectively (RoM 1.155, 0.922–1.447). Both estimates favored melatonin numerically, but the confidence intervals crossed 1, consistent with the primary report that the T2 between-group differences were not statistically significant.

The same raw supplement strengthened the within-study phenotype beyond circulating biomarkers. Relative to CPB, melatonin was associated with lower myocardial ROS (RoM 0.624, 95% CI 0.523–0.744), TUNEL-positive apoptosis (0.241, 0.148–0.391), Bax (0.424, 0.318–0.566), cytochrome c (0.606, 0.395–0.929), cleaved caspase-9 (0.495, 0.286–0.854), AIF (0.790, 0.681–0.916) and LC3 (0.454, 0.318–0.648), while Bcl-2 (2.206, 1.408–3.455) and p62 (3.737, 2.229–6.265) were higher. Phosphorylated AKT, STAT3 and mTOR ratios also increased with melatonin, but these signaling changes are classified as intervention-associated because the study did not perturb these pathways to test whether they were required for protection.

3.4 Mechanistic evidence is model-specific

Perturbation-supported evidence was not distributed uniformly across models. In myocardial I/R, cardiomyocyte-targeted PER2 knockdown *in vivo* and Per2 siRNA *in vitro* attenuated the stronger protection observed when melatonin was administered at the dark-phase time point, supporting a PER2-dependent component and identifying circadian timing as a biological modifier (Luo *et al*., 2025).

In doxorubicin injury, several independent perturbation experiments converged on mitochondrial/redox and regulated cell death pathways without establishing a single universal cascade. AMPK/PGC-1α interference weakened protection in the acute model of Liu *et al*. (2018). YAP siRNA abolished the protective phenotype in Li *et al*. (2020), and YAP down-regulation by siRNA or verteporfin abolished protection against apoptosis, mitochondrial lipid peroxidation and ferroptosis in Sun *et al*. (2022), supporting a YAP/ACSL4-related mechanism in that model. In a separate study, inhibition of either SIRT1 or Nrf2 reversed protection against oxidative stress, pyroptosis and apoptosis (Zhang *et al*., 2023).

Arinno *et al*. (2021) added complementary *in vivo* evidence at the organelle and functional levels. The full primary report randomized 32 male Wistar rats to saline control (n=8) or doxorubicin exposure (n=24); doxorubicin-treated animals were subdivided into vehicle, melatonin and metformin groups (n=8/group). Doxorubicin was administered as six 3 mg/kg intraperitoneal doses on days 0, 4, 8, 15, 22 and 29 (cumulative dose 18 mg/kg), while melatonin was given by oral gavage at 10 mg/kg/day for 30 consecutive days. Echocardiography was performed on day 33 and invasive pressure-volume analysis on day 34. Relative to doxorubicin alone, melatonin improved LVEF, LVFS, E/A ratio, LVESP, dP/dtmax, dP/dtmin and stroke volume and lowered LVEDP and the LF/HF ratio.

It also reduced cTnI, NT-proBNP, serum and cardiac MDA, TNF-α and IL-6; improved mitochondrial respiratory control and membrane potential; decreased mitochondrial swelling and ROS; restored Mfn1, Mfn2, OPA1, PGC-1α and Complex I; attenuated excessive Beclin-1/p62/LC3-II/I signaling; and reduced Bax/Bcl-2, cleaved/pro-caspase-3 and TUNEL positivity. These findings strengthen the mitochondrial-preservation theme but remain intervention-associated rather than pathway-dependency evidence because no genetic or pharmacological perturbation tested whether these molecular changes were required for protection. The article reports bar-chart data as mean ± SEM, with n=5–8/group for functional, injury-marker and mitochondrial-function panels and n=4–6/group for several protein-expression/apoptosis panels. Because exact endpoint-specific sample sizes and group means are not numerically tabulated, no RoM or confidence interval was calculated for this study.

Arinno *et al*. (2023) extended this intervention-associated mitochondrial phenotype to trastuzumab cardiotoxicity. Thirty-two male Wistar rats were allocated to control (n=8) or trastuzumab exposure (n=24); trastuzumab-treated animals were subdivided into vehicle, melatonin and metformin groups (n=8/group). Trastuzumab was administered at 4 mg/kg/day intraperitoneally for 7 days, while melatonin was given orally at 10 mg/kg/day for the same period. Melatonin improved LVEF, LVFS and E/A ratio, mitigated adverse changes in blood pressure, LVESP, LVEDP, stroke volume and sympathovagal balance, and reduced cTnI and NT-proBNP. It was also associated with lower TNF-α, IL-6 and MDA, restoration of GPx4, reduced mitochondrial ROS, improved membrane potential, reduced mitochondrial swelling, increased PGC-1α, more favorable Mfn2/OPA1 and p-Drp1Ser616/Drp1 profiles, improved autophagy markers and reduced apoptosis. Most figure-based endpoints were reported as mean ± SEM with n=5–8/group, while mitochondrial-dynamics assays used n=4–6/group. Because exact group means and dispersions were not numerically tabulated and no raw dataset was available in the primary report, no RoM was calculated; the study is retained as multi-domain intervention-associated evidence rather than pathway-dependency evidence.

After myocardial infarction, endothelial LRRC8A inhibition/knockdown and endothelial-specific deletion eliminated melatonin-associated angiogenic and systolic benefits, supporting an endothelial LRRC8A–AKT/eNOS/HIF-1α-dependent mechanism in that setting (Jie *et al*., 2026). In diabetic cardiomyopathy, Nrf2 genetic ablation and pharmacological inhibition of MT2, SIRT1 or Nrf2 substantially diminished protection, supporting an MT2/SIRT1/Nrf2-dependent contribution (Liu *et al*., 2026).

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By contrast, the 2026 cross-model study by Prestes *et al.* provided Level 2 rather than dependency evidence for two markers. NLRP3 expression decreased in the isoproterenol model together with improved left-ventricular function, whereas xanthine oxidase decreased in monocrotaline-induced pulmonary arterial hypertension together with improved right-ventricular function. These findings are intervention-associated; NLRP3 must not be assigned to the PAH arm, and xanthine oxidase must not be assigned to the isoproterenol arm.

3.5 Historical anthracycline evidence and cohort provenance

Historical anthracycline evidence is no longer uniformly qualitative. Recovery of complete primary reports now provides quantitative data from Sahna, Öz, Agapito and Othman in addition to the previously verified Balli, Bilginoğlu, Liu, Zhang, Aygun and Pehlivan datasets. Agapito contributes directly tabulated cardiac GSSG and GSH values; Othman contributes plasma injury markers and cardiac redox outcomes; and Xu contributes one exact hemodynamic contrast while most of its remaining values are graph-only. Dziegiel *et al*. remains narrative because its MDA+4-HDA, GSH and antioxidant-enzyme results are presented graphically without exact group means; under the primary-source verification rule, those bars were not digitized merely to increase the quantitative study count. Multi-arm shared controls in Sahna and Öz remain non-independent and are not duplicated.

Primary-source verification also clarified the identity and correction history of several studies. The 2001 rat study using melatonin at 6 mg/kg for 15 days was confirmed as Xu *et al.* The 2023 corrigendum to Arinno et al. replaced representative Western-blot images for p62 in Figure 6D and pro-caspase-3 in Figure 7C, without altering the study conclusions. Likewise, the 2024 corrigendum to Liu *et al*. corrected an electron-microscopy panel in Figure 6E and stated that the interpretation and conclusions were unaffected. The complete Liu *et al*. (2018) report therefore remains suitable for reporting the exact *in vivo* apoptosis contrast and for supporting AMPK/PGC-1α as perturbation-supported mechanistic evidence.

Although Pehlivan *et al*. (2020) and Durdagi *et al*. (2021) share the same ethics approval, core author group, doxorubicin challenge, melatonin regimen, and day-8 ECG framework, differences in final group sizes, experimental conditions, comparator arms, assay procedures, and observed outcomes support their treatment as distinct experimental runs within the same project framework. Nevertheless, complete independence cannot be established without animal-level identifiers. Durdagi *et al*. (2021) was therefore retained as a separate qualitative study, while its graph-only biochemical outcomes were not digitized for quantitative comparison. For Arinno *et al*. (2021), recovery of the complete primary article confirms a directly relevant melatonin-versus-doxorubicin comparison, but most outcome panels still report endpoint-specific *n* only as ranges and do not tabulate exact group means; therefore, no graph-derived RoM was calculated.

4. Discussion

The clearest finding of this review is not a single numerical effect but a pattern: across several experimental etiologies, melatonin generally shifted functional, injury-marker and redox outcomes in a cardioprotective direction. The magnitude of that shift was highly variable. Infarct size after I/R, troponins after isoproterenol, biochemical injury during endotoxemia, redox injury in diabetes, myocardial markers during anthracycline exposure and the raw-data-derived CPB biomarkers all moved favorably, yet the corresponding RoMs spanned a wide range. The CPB dataset is particularly illustrative: CK-MB showed a large relative shift, while T2 LVEF and LVFS remained imprecise. This is precisely why an "overall melatonin cardioprotection effect" would be biologically misleading. The initiating lesions, treatment schedules, tissues, assays and ventricular phenotypes are too different for a single pooled number to have a clear physiological interpretation.

Anthracycline cardiotoxicity provides the densest quantitative and mechanistic evidence. Primary-source recovery materially strengthened the historical layer: Sahna provides direct MDA estimates for pre- and post-doxorubicin melatonin; Agapito provides cardiac glutathione redox data; Othman adds independently tabulated CK-MB, LDH, TBARS and GSH; Öz provides sequence-specific MDA, GSH and CPK-MB; and the corrected extraction from Pehlivan et al. adds injury-marker, lipid-peroxidation, and inflammatory outcomes. Together with Balli, Bilginoğlu, Zhang, Liu, Aygun and Xu, these studies show that the protective direction is recurrent but not numerically uniform. This heterogeneity is biologically plausible because anthracycline dose, treatment duration, melatonin schedule, matrix and endpoint definition differ substantially among experiments.

Redox preservation is the most recurrent biological theme across the evidence base, but it should not be equated with a single antioxidant mechanism. Melatonin was associated with lower MDA/TBARS, LPO, 4-HNE or GSSG and with preservation or elevation of GSH, SOD or GPx in multiple models. The newly recovered historical data sharpen this pattern: Agapito showed a marked reduction in cardiac GSSG, Othman showed lower cardiac TBARS with restoration of GSH, Sahna showed lower MDA with both pre- and post-doxorubicin pharmacological melatonin, Öz showed lower MDA with both treatment sequences but a stronger GSH response when melatonin followed doxorubicin, and Zhang showed lower LPO with higher SOD and GSH-Px. Pehlivan is an instructive exception to simplistic antioxidant labeling: its corrected Table 4 confirms lower MDA and inflammatory cytokines, but SOD did not increase in the melatonin arm. The redox phenotype is therefore better interpreted as restoration of oxidative balance than as uniform up-regulation of every antioxidant enzyme.

Mitochondrial preservation appears particularly important in toxic and resuscitation-related injury. Yang *et al*. linked melatonin to suppression of AMPKα2-dependent mitochondrial damage in H9c2 cells, whereas Liu *et al*.

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(2018) supported an AMPK/PGC-1α-dependent contribution to protection in acute doxorubicin injury through both siRNA and *in vivo* Compound C experiments; the same primary report now contributes a directly quantified *in vivo* apoptosis reduction. *In vivo*, the complete primary report by Arinno et al. (2021) showed that a 30-day melatonin regimen during cumulative doxorubicin exposure was accompanied by better LV performance, improved mitochondrial respiratory control and membrane polarization, lower mitochondrial ROS and reduced mitochondrial swelling, and normalization of fusion/fission, biogenesis and Complex I-related bioenergetic markers. Because pathway dependency was not experimentally perturbed in Arinno, these changes remain intervention-associated rather than causal. The 2023 trastuzumab study extended this mitochondrial phenotype to a targeted-therapy injury model. The mechanistic message is therefore not that one mitochondrial pathway explains every model, but that maintenance of mitochondrial integrity is a recurring node reached through different upstream signals.

Inflammatory modulation was also evident but varied by context. The corrected Pehlivan table provides primary numerical support within anthracycline injury: IL-1, IL-6 and IL-18 were all substantially lower with melatonin, while TNF-α was less precise. In the diabetic model, TNF-α, IL-6 and IL-1β were lower while IL-10 was higher. In the CPB study, raw T2 data showed lower IL-6 (RoM 0.529) and a marked reduction in IL-1β (0.080) with melatonin; these are within-study contrasts and should not be interpreted as a pooled inflammatory effect. The trastuzumab model similarly showed lower TNF-α and IL-6, while LPS/endotoxemia studies reported lower inflammatory and nitrosative-stress markers. NLRP3 in isoproterenol injury and xanthine oxidase in PAH remain intervention-associated because pathway dependency was not tested.

Regulated cell death provides another point of convergence. The complete Liu *et al*. (2018) report now adds a directly quantified *in vivo* apoptosis contrast, with TUNEL positivity reduced from 20.68% to 7.32% under melatonin, while AMPK blockade attenuated the broader protective phenotype. Apoptosis markers also improved in diabetes and several other toxic-injury models, while doxorubicin studies implicated pyroptosis and ferroptosis. The trastuzumab study reported less TUNEL positivity and lower pro-apoptotic Bax, cleaved caspase-3 and cytochrome c with melatonin. Huang *et al*. provide quantitative within-study support in CPB as well: TUNEL staining, Bax, cytochrome c, cleaved caspase-9 and AIF all decreased, whereas Bcl-2 increased. The strongest causal evidence still comes from perturbation experiments: YAP down-regulation abolished protection against ferroptosis-related phenotypes, and SIRT1/Nrf2 inhibition reversed melatonin-associated protection against oxidative stress, pyroptosis and apoptosis.

Treatment timing may be as important as dose. Luo *et al*. demonstrated stronger protection at ZT13 than ZT1 and showed loss of protection after PER2 interference, making circadian phase a biologically supported modifier rather than a procedural detail. The recovered historical anthracycline data add a different timing signal: Sahna *et al*. found similar MDA lowering when pharmacological melatonin was started before or after doxorubicin in intact rats, whereas Öz *et al*. observed a more complete biochemical and ultrastructural phenotype when doxorubicin preceded the melatonin course than in the reverse sequence. These studies use different designs and should not be compared as if they tested the same timing question, but together they argue against treating schedule as incidental.

Cardiac compartment also matters. Most studies focused on the left ventricle or systemic myocardial injury, but the monocrotaline model addressed pulmonary arterial hypertension and right-ventricular dysfunction. Pooling LV and RV outcomes would obscure different loading conditions and pathophysiology. The CPB model adds another caution: raw-data-derived injury, inflammatory and redox markers shifted strongly in a protective direction, whereas the T2 LVEF and LVFS estimates were favorable but imprecise and their confidence intervals included 1. Biochemical protection should therefore not be equated automatically with proven functional recovery.

This review has important limitations. It was developed as a structured critical narrative review rather than through a prospectively registered, exhaustive systematic-review workflow; study identification was targeted and iterative, no complete screening log was maintained, and a formal study-level risk-of-bias matrix was not generated. The evidence landscape should therefore be interpreted as a rigorously primary-source-verified account of the retrieved experimental literature, not as proof of exhaustive literature capture. The RoMs, confidence intervals and normalized Prism-style panels are descriptive study-level comparisons and must not be interpreted as pooled estimates or as a common melatonin effect across etiologies. Many experiments used small groups, often five to eight animals per condition, and reporting of randomization, blinding and other design safeguards was not uniformly available.

Several outcomes were reported in only one or two independent experiments, and some studies remain qualitative because exact primary group-level statistics were unavailable or presented only as graphs. This includes Dziegiel and the 2023 trastuzumab study; Durdagi is retained as a distinct qualitative report after full-text comparison with Pehlivan, but its biochemical bars were not digitized. Multi-arm studies introduced an additional constraint because shared injury controls could not be duplicated, and selecting a single arm after observing effect sizes could introduce post hoc bias. Differences in species, sex, injury severity, route, dose, treatment timing, assay matrix and follow-up further limit direct comparability. The common ethics approval and closely related protocols of Pehlivan and Durdagi also illustrate why project-level provenance should be documented explicitly even when the available methods support separate experimental runs.

Human evidence was considered only as translational context and was kept separate from the preclinical quantitative

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comparisons. A 2025 triple-blind placebo-controlled randomized trial in 63 patients with non-metastatic breast cancer receiving doxorubicin plus cyclophosphamide tested 10 mg of melatonin. Post-intervention LVEF was numerically higher without a significant between-group difference, whereas global longitudinal strain and cTnI favored melatonin; CK-MB was lower without statistical significance (Mousavinia *et al*., 2025). This single trial supports continued clinical evaluation but does not establish cardioprotective efficacy in patients and should not be combined with animal effect estimates.

Taken together, the evidence is strongest when cardioprotection is framed as a reproducible but context-dependent phenotype. The recurring biological motifs (redox control, mitochondrial preservation, inflammatory modulation and regulated cell death restraint) are supported across models, while direct perturbation studies identify specific dependencies only in defined settings. The trastuzumab study broadens the cancer-therapy context and reinforces mitochondrial, redox, inflammatory and apoptosis-related consistency without providing a new pooled effect. The Huang dataset also shows the value of author-supplied individual observations: recovery of the raw supplement converted several previously qualitative CPB endpoints into verified study-level effects without graph digitization. Future experiments would be more informative if they prospectively harmonized cardiac functional endpoints, reported raw numerical data with explicit units and dispersion definitions, prespecified timing relative to the light-dark cycle and injury exposure, and incorporated pathway perturbation together with clinically interpretable functional outcomes.

5. Conclusion

Across the experimental literature examined in this review, melatonin consistently shifted cardiac injury toward a more favorable phenotype across ischemic, toxic, metabolic, inflammatory, pulmonary vascular, extracorporeal circulation, and post-resuscitation models. This consistency, however, should not be interpreted as evidence of a single universal cardioprotective effect. The magnitude of benefit varied substantially according to the injury model, experimental design, cardiac compartment, treatment regimen, and measured endpoint. Primary-source quantitative comparisons nevertheless showed recurring reductions in myocardial injury biomarkers, lipid peroxidation, inflammatory mediators, and apoptosis-related signals, together with preservation of ventricular performance and selected components of endogenous antioxidant defense. The anthracycline literature was particularly informative, providing convergent biochemical, functional, histological, mitochondrial, and cell-death evidence while also demonstrating that individual antioxidant enzymes do not necessarily respond in parallel.

The broader dataset further indicates that melatonin-associated cardioprotection is not restricted to a single biochemical pathway. In cardiopulmonary bypass, author-provided raw data showed concordant changes across myocardial injury, inflammation, redox balance, and apoptosis-related outcomes, although the magnitude and certainty of echocardiographic improvement remained more limited. Similar protective patterns were observed in trastuzumab-associated cardiotoxicity, diabetic cardiomyopathy, sepsis and endotoxemia, myocardial infarction, pulmonary arterial hypertension, and post-resuscitation myocardial dysfunction.

Importantly, the strongest mechanistic evidence came from studies in which pathway disruption attenuated or abolished protection. These experiments support model-specific contributions from PER2 in circadian-dependent ischemia/reperfusion protection, AMPK/PGC-1α and YAP/ACSL4 signaling in doxorubicin cardiotoxicity, SIRT1/Nrf2-related signaling in anthracycline injury, endothelial LRRC8A–AKT/eNOS/HIF-1α signaling after myocardial infarction, and MT2/SIRT1/Nrf2 signaling in diabetic cardiomyopathy. Such findings favor a view of melatonin as a pleiotropic modulator of cardiac stress responses rather than as an intervention acting through a single conserved mechanism.

Taken together, the available experimental evidence supports a reproducible, although context-dependent, cardioprotective pattern characterized by attenuation of oxidative injury, inflammatory activation, mitochondrial dysfunction, and regulated cell death, accompanied in many models by preservation of cardiac structure and function. At the same time, heterogeneity in species, injury mechanisms, melatonin dose, route, timing, treatment duration, cardiac region, and outcome definition precludes a universal effect estimate and requires model-aware interpretation of individual results. The study-level quantitative comparisons presented here should therefore be understood as descriptive measures of relative treatment effects rather than as a pooled estimate across etiologies.

Clinical translation remains considerably less certain than the experimental evidence. The available randomized human data provide an initial indication that some of the myocardial-protective signals observed experimentally may be detectable during anticancer therapy, but the clinical evidence remains too limited to establish whether melatonin can meaningfully prevent treatment-related cardiac dysfunction. Larger, independently replicated, and adequately powered clinical studies with standardized cardiovascular endpoints are required before the experimental cardioprotective phenotype can be translated into routine clinical practice. Until such evidence is available, melatonin is best regarded as a biologically plausible and experimentally well-supported cardioprotective candidate whose clinical role remains to be defined.

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