Melatonin and the cardiovascular system in animals: systematic review and meta-analysis

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BACKGROUND

Melatonin, a hormone released by the pineal gland, demonstrates several effects on the cardiovascular system. Herein, we performed a systematic review and meta-analysis to verify the effects of melatonin in an experimental model of myocardial infarction. We performed a systematic review according to PRISMA recommendations and reviewed MEDLINE, Embase, and Cochrane databases. Only articles in English were considered. A systematic review of the literature published between November 2008 and June 2019 was performed. The meta-analysis was conducted using the RevMan 5.3 program provided by the Cochrane Collaboration. In total, 858 articles were identified, of which 13 were included in this review. The main results of this study revealed that melatonin benefits the cardiovascular system by reducing infarct size, improving cardiac function according to echocardiographic and hemodynamic analyses, affords antioxidant effects, improves the rate of apoptosis, decreases lactate dehydrogenase activity, enhances biometric analyses, and improves protein levels, as analyzed by western blotting and quantitative PCR. In the meta-analysis, we observed a statistically significant decrease in infarct size (mean difference [MD], -20.37 [-23.56, -17.18]), no statistical difference in systolic pressure (MD, -1.75 [-5.47, 1.97]), a statistically significant decrease in lactate dehydrogenase in animals in the melatonin group (MD, -4.61 [-6.83, -2.40]), and a statistically significant improvement in the cardiac ejection fraction (MD, -8.12 [-9.56, -6.69]). On analyzing potential bias, we observed that most studies presented a low risk of bias; two parameters were not included in the analysis, and one parameter had a high risk of bias. Melatonin exerts several effects on the cardiovascular system and could be a useful therapeutic target to combat various cardiovascular diseases.

KEYWORDS: Cardiology; Melatonin; Meta Analysis; Review; Systematic Review.

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to verify the effects of melatonin in an experimental model of myocardial infarction.

SEARCH STRATEGIES

In the present study, the search strategy was performed as described by Tawfik et al. (15). We used MEDLINE, Google Scholar, and Cochrane databases and reviewed literature published from November 2008 to June 2019; we restricted this systematic review to the last ten years, covering the latest and most relevant articles worldwide. First, we selected keywords from related articles, using Medical Subject Headings (MeSH) to identify more related keywords with similar meanings as follows: (“melatonin”) [MeSH Terms] AND (“cardiovascular system”) [MeSH Terms] [All Fields]. We then searched the three databases. Accordingly, we identified 2096 articles in PubMed using the “other animals” filter, 602 articles using Google Scholar filtering for keywords only in the title, and three articles using a Cochrane Library advanced search; the terms used were “melatonin and cardiovascular system” In addition, we reviewed retrieved articles to identify additional studies (Figure 1). This review was conducted according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (16,17).

We excluded studies with cell culture experiments, as well as pre- and post-conditioning studies. The inclusion criteria were animal studies, cell culture studies, and in vivo experiments. The control group was the melatonin group in this study. The melatonin group varied in each article, as studies persistently experimented with a melatonin group related to a drug or an event.

The process of paper retrieval and titles and abstract evaluation was conducted by two independent blinded researchers capable of compiling systematic reviews (ECV and RS), following the inclusion and exclusion criteria.

![Flow chart of experimental design](image)

**Figure 1** - Flow chart of experimental design.
according to the tenets of PICO (16-19). The PICO was defined as patients in case the systematic review was performed in animals, interventions considering the administration of melatonin in animals using an experimental model of myocardial infarction, comparison, to compare the melatonin group with the control group receiving no melatonin, and outcome, which were results of administering melatonin. The selected articles were critically evaluated to determine their potential inclusion in the review. In the event of a disagreement between investigators regarding studies selected, a third reviewer was consulted (LCA).

In the present systematic review, data obtained from selected studies were tabulated, and the following characteristics were listed when present in the articles: authors’ names, year of publication, animal type, sex (M/F), animal species, age (months), weight, induction model, and site injury (Table 1). Table 2 presents the following information: authors, sample size, number of groups, number of animals per group, melatonin administration, melatonin doses, and dependent variables. Table 3 lists the most frequent recommendations in preclinical research guidelines for in vivo animal experiments (18). Table 4 evaluates the study characteristics of selected controlled animal studies, with prior exercise and myocardial infarction as variables that showed a significant difference between the melatonin control group and the study group. These were classified as S for “significant difference,” and variables that did not present a significant difference were classified as NS (not significant).

RevMan (version 5.3; Cochrane Collaboration, Oxford, UK) was used to perform the meta-analysis. The random-effects model was used to account for the heterogeneity.

### Statistical analysis

Mean values and standard deviation between studies, presented as the mean difference (MD) of post-intervention values after calculating the inverse variance, were employed to verify the magnitude of the protection afforded by melatonin (19). In addition, heterogeneity was assessed using Cochran’s Q and I² tests, followed by visual inspection of the graph. The analyses were performed using the RevMan software (version 3.3.1) (20).

## RESULTS

Figure 1 presents the search process, identification, and selection of articles. Based on our search strategies, 73 articles were retrieved from 2099 identified articles in PubMed using the “other animals” filter; among these, 18 were selected after reading the title and abstract. In addition, we selected three articles from BIREME and 0 articles from the Cochrane database. The inclusion and exclusion criteria are described in Figure 1 (21-38). Articles were primarily excluded when assessments were performed in human subjects, apart from being unrelated to components of PICO; we mainly focused on experimental animal studies.

In Table 4, we employed the criteria of Henderson et al. (18). We found that 61.11% (21,22,25-27,30,31,33,35,36,38) of selected studies had an appropriate sample size and 61.11% (21,23,25,26,27,30,31,33-36) had randomized animals, according to their materials and methods. All articles were blinded to the outcome assessment (21-38). We could not determine the criterion underlying the flow of animals through experiments, as no explicit statement regarding the same was available in the materials and methods. We observed that

### Table 1

| Authors          | Animal type | Animal race | Age (months) | Weight | Induction model | Site injury |
|------------------|-------------|-------------|--------------|--------|-----------------|-------------|
| Zhang et al. (21)| Mice C57/B6 | -           | Sepsis-induced cardiac dysfunctional | Cardiovascular system |
| Benova et al. (22)| Rat Wistar | 9 months    | Obesity | Cardiovascular system |
| Chen et al. (23) | Rat Sprague-Dawley | - | 200–250 g Myocardial ischemia reperfusion | Myocardial tissue |
| Liu et al. (24)  | Mice C57/B6 | -           | -           | 250–350 g Myocardial ischemia reperfusion | Heart |
| Simko et al. (25)| Rat Wistar | 3 months    | Hypertension | Myocardial ischemia reperfusion | Heart |
| Liu et al. (26)  | Wistar B6.V-lean/OlaHsd and B6.V-Lepob/OlaHsd | 4 weeks | Obesity | Mitochondria of cardiomyocyte |
| Chaudhary et al. (27)| Rat        | 290–320 g | Myocardial infarction | Heart |
| Repova et al. (28)| Rat Wistar | 300–330 g | Myocardial ischemia reperfusion in vivo | Heart |
| Cheng et al. (29)| Mice Gpx-/- C57BL/6 | - | Myocardial ischemia reperfusion | Heart |
| Petrov et al. (30)| Rat Wistar | - | 250–330 g Myocardial ischemia reperfusion | Heart |

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**Revision:**

- Table 1: Study characteristics of selected control experimental studies assessing melatonin and the cardiovascular system.
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| Authors             | Sample size | Number of groups | Number of animals/groups | Melatonin administration | Melatonin doses | Dependent variables                                                                                                                                 |
|---------------------|-------------|------------------|--------------------------|--------------------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Zhang et al. (21)   | 24          | 4                | 6                        | Intraperitoneal injection | 30 mg/kg       | Echo, histological analysis, creatinine kinase measurement, TUNEL analysis, western blotting, Heart function in Langendorff perfusion, western blot, real-time PCR,   |
| Benova et al. (22)  | 48          | 4                | 12                       | Drinking water           | 10 mg of melatonin was dissolved in 100 mL of water for 8 weeks | Heart function in Langendorff perfusion, western blot, real-time PCR, detection of autophagosomes, Echo, histological analysis, PCR, western blot, CTRP3 detection. |
| Chen et al. (23)    | 30          | 5                |                          | Intraperitoneal at the reperfusion | 20 mg/kg       | Echo, IS2, lactate dehydrogenase release, CMEC measurement in vitro IRI assay, western blotting, qRT-PCR, and detection of autophagosomes.                  |
| Liu et al. (24)     | 18          | 3                | 6                        | Gavage                   | 50 mg/kg       | Hemodynamics measures, biometric analysis, determination of hydroxyproline, angiotensin, aldosterone analysis.                                        |
| Simko et al. (25)   | 40          | 4                | 10                       | Water consumption was 12-13 mL/100 g of body weight | 10 mg of melatonin was dissolved in 100 mL of water for 4 weeks | Hemodynamics measures, determination of hydroxyproline, NO synthase activity, oxidative load measurement, and western blotting of NF-κB.                   |
| Simko et al. (26)   | 66          | 6                | 11                       | Drinking water adjustment to daily water consumption to ensure the correct dosage | 10 mg/kg/day for 6 weeks | Hemodynamics measures, determination of hydroxyproline, NO synthase activity, oxidative load measurement, and western blotting of NF-κB.                   |
| Stacchiotti et al. (27) | 40      | 4                | 10                       | 5th to 13th weeks of life/drinking water | 100 mg/kg/day for 8 weeks | Histomorphometric evaluations, nuclear cardiomyocyte morphometry, mitochondrial and immunohistochemical analysis.                                    |
| Chaudagar et al. (28) | 24          | 4                | 6                        | Drinking water           | 10 mg/kg/day for 67 days | Hemodynamics measures, bioassay, and NO assays.                                                                                                    |
| Salmanoglu et al. (29) | 35         | 6                |                          | Oral gavage              | 10 mg/kg/day for 2 weeks | Vasocontractile response, measurement of total cholesterol, LDL, HDL, glucose, NO, and insulin, MDA assay, and tissue antioxidant levels.                           |
| Cheng et al. (30)   | 60          | 3                | 20                       | -                        | 20 mg/kg for 4 weeks | Immunochemical analysis, HE staining, western blot analysis, and qRT-PCR.                                                                            |
| Liu et al. (31)     | 60          | 5                | 12                       | Intravenous injection immediately after reperfusion | 10 mg/kg       | IF2, myocardial ultrastructure, western blotting and determination of the opening degree of MPTPs.                                                   |
| Zhu et al. (32)     | -           | -                | -                        | Melatonin stem cells were treated for 24 hours | 5 μM           | Measurements of cell culture antioxidant properties, apoptosis, analysis of paracrine factors, LV functions, histology.                                 |
| Liu et al. (33)     | 60          | 6                | 12                       | Intraperitoneal injection | Group I: 2.5 mg/kg, Group II: 5 mg/kg, Group III: 10 mg/kg | Hemodynamics measures, apoptosis, electron microscope examination, analysis on mitochondria.                                                         |
| Drobnik et al. (34) | 21          | 3                | 7                        | Drinking water for 6 weeks | 10 mg/kg       | Collagen determination, estimation of glycosaminoglycans, electron microscopy examination.                                                          |
| Repova et al. (35)  | 40          | 4                | 10                       | Drinking water for 6 weeks | 10 mg/kg       | Collagen determination, hemodynamics measures.                                                                                                    |
| Drobnik et al. (36) | 60          | 5                | 12                       | Intraperitoneal injection for 4 weeks | Group 1: 300 μg/100 g b.w. Group 4: 3 mg/100 g b.w. Group 5: 1.5 mg/100 g b.w. | Estimation of lipid peroxidation, collagen determination, estimation of glycosaminoglycans.                                                        |
| Chen et al. (37)    | -           | -                | -                        | Intraperitoneal injection 30 min before harvesting the heart for in vitro preparation | 150 μg/kg      | Cardiac function, hemodynamics measures, lactate dehydrogenase released, apoptosis, immunohistochemistry.                                           |
| Petrosillo et al. (38) | 42         | 6                | 7                        | Krebs-Henseleit solution for isolated heart | 50 μM          | Infarct size, lactate dehydrogenase released, hemodynamics measures, analysis on mitochondria.                                                    |

IS1, measurement of infarct size by echocardiography; IS2, measurement of infarct size by Evans Blue or tetrazolium; echo, echocardiography measurements; CMEC, cardiac microvascular endothelial cells; IRI, ice recrystallization inhibition; CTRP3, C1q TNF Related Protein 3; NO, nitric oxide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MDA, malondialdehyde; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; LV, left ventricular; MPTP, mitochondrial permeability transition pore; NF-κB, Nuclear factor-kappa B; g.b.w., gross body weight; HE, hematoxylin-eosin.
Table 3 - Most frequent recommendations appearing in preclinical research guidelines for in vivo animal experiments [Hendersen et al. (18)].

| Validity type | Recommendation Category | Examples |
|---------------|-------------------------|----------|
| Internal      | Choice of sample size   | Control of animal properties at baseline |
|               | Randomized allocation of animals | Treatment response according to a mechanistic pathway |
|               | Blinding of outcome assessment | Matching model to the human manifestation of the disease |
|               | Flow of animals through an experiment | Matching model to the age of patients in clinical settings |
|               | Using functional or non-surrogate outcome measures | Characterizing pathway in terms of molecular biology, histology, physiology or behavior |
|               | Independent replication | Inter-study standardization of experimental design |
|               | Replication in different species | |
Table 4 - Most frequent recommendations in preclinical research guidelines for in vivo animal experiments [Henderson et al. (18)].

| Validity type                | Recommendation Category                        | Studies                                                                 | n (Percent of guidelines Citing) |
|------------------------------|-----------------------------------------------|-------------------------------------------------------------------------|---------------------------------|
| Internal                     | Choice of sample size                          | Zhang et al. (21); Benova et al. (22); Simko et al. (25); Simko et al. (26); Stacchiotti et al. (27); Cheng et al. (30); Liu et al. (31); Liu et al. (33); Repova et al. (35); Drobnik et al. (36); Petrosillo et al. (38). | 61.11%                          |
|                              | Randomized allocation of animals to treatment  | Zhang et al. (21); Benova et al. (22); Simko et al. (25); Simko et al. (26); Stacchiotti et al. (27); Cheng et al. (30); Liu et al. (31); Liu et al. (33); Repova et al. (35); Drobnik et al. (36). | 61.11%                          |
|                              | Blinding of outcome assessment                 | Zhang et al. (21); Benova et al. (22); Chen et al. (23); Simko et al. (25); Liu et al. (24); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Cheng et al. (30); Liu et al. (31); Zhu et al. (32); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 100%                            |
| Flow of animals through an experiment | -                                             | -                                                                       | -                               |
| Selection of appropriate control groups | -                                             | Zhang et al. (21); Chen et al. (23); Simko et al. (25); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Cheng et al. (30); Liu et al. (31); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Petrosillo et al. (38). | 77.77%                          |
| Study of dose-response relationships | -                                             | Chen et al. (23); Simko et al. (25); Simko et al. (26); Chaudagar et al. (28); Cheng et al. (30); Liu et al. (31); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Petrosillo et al. (38). | 61.11%                          |
| Construct                    | Characterization of animal properties at baseline | Zhang et al. (21); Benova et al. (22); Chen et al. (23); Simko et al. (25); Liu et al. (24); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Cheng et al. (30); Liu et al. (31); Zhu et al. (32); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 100%                            |
|                              | Matching model to the human manifestation of the disease | Zhang et al. (21); Benova et al. (22); Chen et al. (23); Simko et al. (25); Simko et al. (26); Salmanoglu et al. (29); Cheng et al. (30); Liu et al. (31); Zhu et al. (32); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 88.88%                          |
|                              | Treatment response along a mechanistic pathway | Zhang et al. (21); Benova et al. (22); Chen et al. (23); Simko et al. (25); Liu et al. (24); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Cheng et al. (30); Liu et al. (31); Zhu et al. (32); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 100%                            |
|                              | Matching outcome measures to clinical settings  | Chen et al. (23); Simko et al. (25); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Cheng et al. (30); Liu et al. (31); Zhu et al. (32); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 66.66%                          |
|                              | Matching model to the age of patients in clinical settings | Zhang et al. (21); Benova et al. (22); Chen et al. (23); Simko et al. (25); Liu et al. (24); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Liu et al. (31); Zhu et al. (32); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 100%                            |
| External                     | Replication in different models of the same disease | -                                                                       | -                               |
|                              | Independent replication                          | Zhang et al. (21); Chen et al. (23); Simko et al. (25); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Cheng et al. (30); Zhu et al. (32); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 77.77%                          |
|                              | Replication in different species                 | Zhang et al. (21); Benova et al. (22); Chen et al. (23); Simko et al. (25); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Salmanoglu et al. (29); Liu et al. (31); Zhu et al. (32); Liu et al. (33); Drobnik et al. (34); Repova et al. (35); Drobnik et al. (36); Chen et al. (37); Petrosillo et al. (38). | 88.88%                          |
| Research program             | Inter-study standardization of experimental design | Simko et al. (25); Simko et al. (26); Stacchiotti et al. (27); Chaudagar et al. (28); Zhu et al. (32); Repova et al. (35); Chen et al. (37). | 38.88%                          |
| Type of bias          | Domain                          | Description of domain                                                                 | Review authors judgment                                                                 |
|----------------------|---------------------------------|----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Selection bias       | Sequence generation             | Describe the methods used, if any, to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups. | Was the allocation sequence adequately generated and applied?                             |
| Selection bias       | Baseline characteristics        | Describe all the possible prognostic factors or animal characteristics, if any, that are compared to judge whether intervention and control groups were similar at the start of the experiment. | Were the groups similar at baseline, or were they adjusted for confounders in the analysis? |
| Selection bias       | Allocation concealment          | Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrolment. | Was the allocation adequately concealed?                                                  |
| Performance bias     | Random housing                  | Describe all measures used, if any, to house animals randomly within the animal room.   | Were the animals randomly housed during the experiment?                                   |
| Performance bias     | Blinding                        | Describe all measurements, if any, to blind trial caregivers and researchers from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective. | Were the caregivers and/or investigators blinded from knowledge regarding the intervention each animal received during the experiment? |
| Detection bias       | Random outcome assessment       | Describe whether animals were selected at random for outcome assessment and which methods to select the animals, if any, were used. | Were animals selected at random for outcome assessment?                                   |
| Detection bias       | Blinding                        | Describe all measures used, if any, to blind outcome assessors from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective. | Was the outcome assessor blinded?                                                        |
| Attrition bias       | Incomplete outcome data         | Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized animals), reasons for attrition or exclusions, and any re-inclusions in analysis for the review. | Were incomplete outcome data adequately addressed?                                         |
| Reporting bias       | Selective outcome reporting     | State how selective outcome reporting was examined and what was determined.             | Are reports of the study free of selective outcome reporting?                             |
| Other                | Other sources of bias           | State any important concerns regarding bias not covered by other domains in the tool.  | Was the study free of other problems that could result in a high risk of bias?            |

Figure 2 - Representation of the SYRCLE's risk of bias tool for animal studies. Hooijmans et al. (43).
Table 5 - Study characteristics of selected controlled animal studies assessing melatonin and the cardiovascular system.

| Authors            | Assessments                                                                 |
|--------------------|-----------------------------------------------------------------------------|
| Zhang et al. (21)  | S Echocardiography measurements S Apoptosis analysis S Western blotting S Creatinine kinase measurement S Immunohistochemical analysis NS Detection of autophagosomes |
| Benova et al. (22) | S Biometric analysis S Western blotting S qRT-PCR Hemodynamics measures NS |
| Chen et al. (23)   | S Measurements of the infarct size S Measurement of lactate dehydrogenase S Measures of CMEC in vitro IRI assay S Western blotting S qRT-PCR S Detection of autophagosomes |
| Liu et al. (24)    | S Echocardiography measurements S Hemodynamics measurements S Apoptosis analysis S Western blotting NS |
| Simko et al. (25)  | S Biometric analysis S Hemodynamics measures NS Determination of hydroxyproline S Angiotensin analysis NS Aldosterone analysis |
| Simko et al. (26)  | NS Hemodynamics measures NS Determination of hydroxyproline NS NO synthase activity S Oxidative load S Measurement and western blotting of NF-κB |
| Stacchiotti et al. (27) | S Histomorphometrically evaluations NS Nuclear cardiomyocyte morphometric S Mitochondrial analysis S Immunohistochemical analysis |
| Chaudagar et al. (28) | NS Hemodynamics measures NS Biometric analysis S NO assays S Mitochondrial analysis NS |
| Salmanoglu et al. (29) | NS Vasocontractile response NS Measures of total cholesterol, LDL, HDL NS NO assays S MDA assay NS Measurements of tissue antioxidant levels |
| Cheng et al. (30)  | S Immunohistochemical analysis S Western blotting S qRT-PCR S S |
| Liu et al. (31)    | S Measurements of the infarcted size S Western blotting S Determination of the opening degree of MPTPs |
| Zhu et al. (32)    | S Measurements of cell cultures antioxidant properties S Apoptosis analysis S LV functions |
| Liu et al. (33)    | S Hemodynamics measures S Apoptosis analysis S Electron microscope examination S Analysis on mitochondria |
| Drobnik et al. (34) | S Determination of collagens S Determination of glycosaminoglycans S Electron microscope examination |
| Repova et al. (35) | S Hemodynamics measures S Determination of collagen S Determination of glycosaminoglycans |
| Drobnik et al. (36) | S Estimation of lipid peroxidation S Determination of collagen NS S Determination of glycosaminoglycans |

(Continued)
The meta-analysis revealed a statistically significant decrease in infarct size (MD -20.37 [-23.56, -17.18]). However, there was no statistical difference in systolic pressure between articles analyzed (MD -1.75 [-5.47, 1.97]). In articles analyzing lactate dehydrogenase, a statistically significant decrease in the levels of this enzyme was noted in animals in melatonin groups (MD -4.61 [-6.83, -2.40]). With regard to the ejection fraction, two articles showed improvement in melatonin-treated groups. Another study analyzed the influence of melatonin in infarcted animals with the same ejection fraction; however, this parameter was not statistically significant in the meta-analysis (MD -8.12 [-9.56, -6.69]) (Figure 3).

In terms of selection bias, the results were well-balanced between low risk, no clear risk, and high risk of bias. All studies presented a low risk of bias in the baseline variable characteristics. On analyzing allocation concealment, most selected articles had a high risk, and a little less than half presented a low risk of bias. The randomization parameter was also fairly balanced between low risk, no clear risk, and high risk of bias. On analyzing random outcome assessment, most studies (more than 50%) had a low risk of bias, and some presented an unclear risk of bias. On analyzing blinding bias, most articles were unclear as to whether investigators were blinded. The articles presented a low risk of bias in the results of incomplete outcome data (Figure 4 and 5).

**DISCUSSION**

This systematic review revealed that melatonin has various beneficial effects on the cardiovascular system; these effects include decreased infarct size, improved cardiac function and cellular oxidation functions, reduced apoptosis, and healthier cellular histomorphology.

In the present review, studies that analyzed echocardiographic measures exhibited melatonin benefits such as decreased infarct size, improved ejection fractions, improved systolic and diastolic diameters, and ameliorated recovery rates of cardiac function (24), while also favoring the treatment of cardiac hypertrophy and hearts that experienced myocardial infarction, ischemia, or reperfusion (21-38). On analyzing hemodynamic and biometric variables, melatonin appeared to confer significant benefits, such as improvements in systolic pressure, positive pressure derivative, lower left ventricular end-diastolic pressure, reduced left ventricular weight in relation to the total heart weight, and improved lung water content (22,25,28,35,38). Experimental models of obesity, hypertension, and other cardiovascular diseases reinforce the scientific practice of adopting animal models and assessing results prior to human application. These preclinical results indicate the effect of melatonin on the examined cardiovascular diseases.

Reportedly, melatonin is an important anti-apoptotic agent in various tissues, reducing calcium uptake, mitigating reactive oxygen species generation, and decreasing the levels of pro-apoptotic proteins, such as Bax (39). In addition, melatonin destabilizes hypoxia-induced hypoxia-inducible factor (HIF)-1α protein expression. Moreover, melatonin suppresses HIF-1α transcriptional activity under hypoxic conditions, resulting in vascular endothelial growth factor expression (40). Melatonin also confers anti-inflammatory effects on the cardiovascular system (41). Furthermore, a systematic review and recent meta-analysis have identified
Figure 3a - Metanalysis of infarct size measurement by echocardiography (% left ventricular).

Figure 3b - Metanalysis of systolic blood pressure (mmHg).

Figure 3c - Metanalysis of lactate dehydrogenase (U/L).

Figure 3d - Metanalysis of ejection fraction measured by echocardiography (% left ventricular).

Figure 4 - Risk of bias graph: review of authors’ judgment regarding each risk of bias item presented as percentages across all included studies.
that melatonin supplementation facilitates blood pressure regulation (42).

Melatonin has substantial benefits in the heart, involving various proteins (including superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [Gpx]), while also improving the apoptosis rate. These findings were determined using several techniques, including western blotting analysis of BCL and Bx expression and the TUNEL assay, which measured the decrease in the level of apoptosis in myocardial cells when melatonin was added (23-25,29,32). Other important variables analyzed following melatonin administration in the cardiovascular system were lactate dehydrogenase levels, mitochondrial analysis, lipid peroxidation, glycosaminoglycan, collagen level reduction, culture measurements, the antioxidant action of cells, opening gradient of mitochondrial channels, improvement in vasoconstriction, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and cholesterol level measurements, nitric oxide.

Figure 5 - Risk of bias summary: review of authors’ judgment regarding each risk of bias item for each included study.
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CONCLUSION

Notably, this systematic review is based on animal experiments. Melatonin may impact the cardiovascular system, including experimental myocardial infarction, and further studies are necessary to determine its use in clinical settings for treating cardiovascular diseases.

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AUTHOR CONTRIBUTIONS

Veiga ECA contributed substantially to the study conception and design, definition of intellectual content, was involved in literature search, data analysis, statistical analysis, and manuscript preparation, drafting and critical review for important intellectual content, and approved the final manuscript version to be published. Simões RS, Cavalli LL, Abreu LC and Cavalli RC were involved in data analysis and statistical analysis, manuscript drafting and critical review for important intellectual content, and approved the final manuscript version to be published. Cipolla-Neto J, Baracat EC and Soares Junior JM substantially contributed to the study conception and design, definition of intellectual content, were involved in manuscript preparation, drafting and critical review for important intellectual content, and approved the final manuscript version to be published.

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