SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Natural plants in the treatment of experimental myocardial injury: a systematic review [provisional title]	
2.	Authors (names, affiliations, contributions)	**Raquel Moreira de Britto, Msc** Department of Physiology, Universidade Federal de Sergipe. Brazil. RaquelBritto81@gmail.com **Fernando Kenji Nampo**, PhD. Latin-American Institute of Life and Natural Sciences, Universidade Federal da Integração Latino-Americana. Brazil and Department of Physical Therapy, Universidade Federal de Sergipe. Brazil. Fernando.Nampo@gmail.com **Vinícius Cavalheri,** PhD. School of Physiotherapy and Exercise Science Curtin University, Australia V_Cavalheri@hotmail.com **David Nascimento**, BSc. Physical Therapy Department, Filadelfia University Center, Brazil david-htp@hotmail.com **Nara Michelle Moura Soares, Msc** Physical Education Department Tiradentes University. Brazil Narasoares963@hotmail.com **Enilton Aparecido Camargo**, PhD. Department of Physiology, Universidade Federal de Sergipe. Brazil. Enilton.Camargo@gmail.com **Sandra Lauton Santos**, PhD. Department of Physiology, Universidade Federal de Sergipe. Brazil. SandraLauton@gmail.com	
3.	Other contributors (names, affiliations, contributions)	N/A	
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6.	Conflicts of interest	Authors affirm that we have no financial affiliation or involvement with any commercial organization that has a direct financial interest in any matter included in this research.	
7.	Date and location of protocol registration	December 11th 2015, SYRCLE	
8.	Registration number (if applicable)	N/A	
9.	Stage of review at time of registration	Not started.	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	According to DATASUS data, about 66.000 victims of acute myocardial infarction (AMI) die each year in Brazil. It is considered the greatest single cause of death in the country. Since the estimate of cases is 300 to 400 thousand cases a year, the death rate is extremely high. Reperfusion is used as a means of intervention for acute AMI. However, reperfusion has the potential to exacerbate the tissue damage a designated process "reperfusion injury", accounting for 50% of infarct size. Reperfusion injury is represented by abnormalities such as arrhythmias, mechanical dysfunction or "stunning myocardium", microvascular injury, inflammatory responses and apoptosis. This systematic review will compile preclinical research on natural plants investigated in the treatment of myocardium reperfusion injury and, thereafter, offer future perspectives in this field.	
	Research question		
11.	Specify the disease/health problem of interest	Acute myocardial infarction (AMI)	
12.	Specify the population/species studied	Animals submitted to AMI either surgically of not.	
13.	Specify the intervention/exposure	Natural plants used both on in vivo or ex-vivo experimentation.	
14.	Specify the control population	Control group (placebo, sham treatment).	
15.	Specify the outcome measures	Biochemical Parameters: ✓ Lipid Peroxidation-TBARS, ✓ Total Hydroperoxide, ✓ Superoxide dismutase (SOD)	

		✓ Catalase (CAT) ✓ Glutathione peroxidase (GPx) ✓ Glutathione reductase (GR) ✓ Creatine kinase (CK) and isoenzyme (CK-mb) ✓ Lactate dehydrogenase (LDH) Molecular parameters: ✓ Caspase 3 ✓ Bax ✓ Bcl Electrophysiological parameters and contractile ✓ left intraventricular pressure (PVE), ✓ systolic pressure of the left ventricle (PSVE) ✓ end-diastolic pressure of the left ventricle (PDFVE) ✓ maximum positive derivative of the pressure VE (+dP/dtmax) ✓ maximum negative derivative of the pressure VE (-dP/dtmax) ✓ Frequency cardiac, ✓ Complex QRS ✓ Break QTC ✓ Break RR ✓ ST (super or depression) Echocardiographic parameters: ✓ Left ventricle ✓ Measurements of the cavity of the left ventricle in diastole (DDVE) and systole (DSVE) and ejection fraction (FE) Histology: ✓ Analysis of the area of infarction	
16.	State your research question (based on items 11-15)	Compared to the controls, are natural plants effective in protecting cardiac muscle against reperfusion injury following AMI?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	✓ MEDLINE via PubMed ✓ SCOPUS ✓ EMBASE ✓ SCIELO ✓ LILACS ✓ SCISEARCH via DIMDI	

		✓ Other, namely: Grey literature (Google Scholar)	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	Simplified search strategy: Natural plants: ethnobotan*OR Ethnopharmacolog* OR ethno botan* OR caatinga OR inner bark OR traditional chinese medicine OR chinese medicine OR chinese medicine OR natural products OR natural product OR plant OR plants OR phytother* Ischaemia Reperfusion injury 'ischemia reperfusion' OR 'ischemia'[MeSH terms] OR 'postoperative stress' OR 'perioperative stress' OR 'ischaemia injury' OR 'ischaemia reperfusion' Myocardium/heart Cardiac OR heart OR myocardial OR myocard* Animals: Filter for animal studies	
19.	Identify other sources for study identification	☑Reference lists of included studies □Books ☑Reference lists of relevant reviews □Conference proceedings, namely: □Contacting authors/ organisations, namely: □Other, namely:	
20.	Define search strategy for these other sources	Google scholar, Google.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1. Title/abstract screening. 2. Full text screening.	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	a. Two reviewers will independently screen for relevant studies. b. Discrepancies will be resolved either by discussion or by a third reviewer (when no agreement is met by the two reviewers).	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: Pre-clinical study Exclusion criteria: There will be no exclusion criterion	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: laboratory animal with AMI. Exclusion criteria: Animals with pre-established disease	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Natural plants independently of timing of treatment.Exclusion criteria: Association with other interventions	
26.	Outcome measures	Inclusion criteria: Biochemical ,molecular,	

		electrophysiological, contractile, and echocardiographic parameters. Measurement of infarct area Exclusion criteria: There will be no exclusion criterion	
27.	Language restrictions	Inclusion criteria: No restriction. Exclusion criteria: There will be no exclusion criterion	
28.	Publication date restrictions	Inclusion criteria: Studies published up to search date. Exclusion criteria: No past date restriction.	
29.	Other	Inclusion criteria: N/A. Exclusion criteria: No original paper (e.g. review).	
30.	Sort and prioritize your exclusion criteria per selection phase	abstract screening. 1. Type of study. 2. Type of animals. 3. Type of intervention. Selection phase: Full text screening. 1. Type of study. 2. Type of animals. 3. Type of intervention. 4. Outcome measures.	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, title, year, language, contact author e-mail	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Experimental groups. Number of animals per group.	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Animal species, strain, age or weight, gender. Induction for myocardial ischemia technique	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Type of analgesics, Route of administration, dose (natural plant investigated), frequency, timing relative AMI induction, duration of treatment, type of control group	
35.	Outcome measures	Biochemical Parameters: ✓ Lipid Peroxidation-TBARS, (nmol de MDA/mg de tecido); ✓ Total Hydroperoxide (mol/ L); ✓ Superoxide dismutase (SOD) (U/mg de proteína); ✓ Catalase (CAT) (ΔE/min/mg de proteína); ✓ Glutathione peroxidase (GPx) (nmol NADPH/min/mg proteína); ✓ Glutathione reductase (GR)(mU/min/mg proteína) ✓ Creatine kinase (CK) and isoenzyme (CK-mb) (U/L) ✓ Lactate dehydrogenase (LDH) (U/L) Molecular parameters: ✓ Caspase 3 (u.a) ✓ Bax (u.a) ✓ Bcl (u.a)	

		Electrophysiological parameters and contractile ✓ left intraventricular pressure (PVE), (cmHg) ✓ systolic pressure of the left ventricle (PSVE) (mmHg) ✓ end-diastolic pressure of the left ventricle (PDFVE) (mmHg) ✓ Heart rate (bpm) ✓ Complex QRS (mm) ✓ Break QTC (mm) ✓ Break RR(mm) Echocardiographic parameters: ✓ Measurements of the cavity of the left ventricle in diastole (DDVE) (cm) and systole (DSVE) (cm) and ejection fraction (FE) (%) Histology: Analysis of the area of infarction (%)	
36.	Other (*e.g.* drop-outs)	Country of origin. Age of sacrificing animals, anesthetics used for sacrificing	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	a. Two reviewers will independently evaluate risk of bias of included studies. b. Discrepancies will be resolved either by discussion or by a third reviewer (when no agreement is met by the two reviewers).	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	✓ By use of SYRCLE's Risk of Bias tool[4] ☐By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐By use of CAMARADES' study quality checklist, e.g [22] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Biochemical Parameters: ✓ Lipid Peroxidation-TABRS, (Continuous) ✓ Total Hydroperoxide, (Continuous) ✓ Superoxide dismutase (SOD) (Continuous) ✓ Catalase (CAT) (Continuous) ✓ Glutathione peroxidase (GPx) (Continuous) ✓ Glutathione reductase (GR) (Continuous) ✓ Creatine kinase (CK) and isoenzyme (CK-mb) (Continuous) ✓ Lactate dehydrogenase (LDH) (Continuous) Molecular parameters: ✓ Caspase 3 (Continuous)	

		✓ Bax (Continuous) ✓ Bcl(Continuous) Electrophysiological parameters and contractile ✓ left intraventricular pressure (PVE), (Continuous ✓ systolic pressure of the left ventricle (PSVE) (Continuous) ✓ end-diastolic pressure of the left ventricle (PDFVE) (Continuous) ✓ maximum positive derivative of the pressure VE (+dP/dtmax) (Continuous) ✓ maximum negative derivative of the pressure VE (-dP/dtmax) (Continuous) ✓ Frequency cardiac, (Continuous) ✓ Complex QRS (Continuous) ✓ Break QTC (Continuous) ✓ Break RR (Continuous) ✓ ST (super or depression) (Continuous) Echocardiographic parameters: ✓ left ventricle (dichotomous) ✓ measurements of the cavity of the left ventricle in diastole (DDVE) and systole (DSVE) and ejection fraction (FE) (dichotomous) Histology: ✓ Analysis of the heart attack (dichotomous)	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	Data will be extracted preferably from published data (explicit numeral). Whenever necessary, an electronic mail will be send to the correspondent author for further information. If no answer is obtained within a week or there is no contact information, other authors will be randomly contacted. After five weeks, if no answer is received, the study will be excluded from analysis.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	a. Two reviewers will independently extract data from included studies. b. Discrepancies will be resolved either by discussion or by a third reviewer (when no agreement is met by the two reviewers).	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	To all outcomes meta-analysis is intended.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	To all outcomes: - At least two studies.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	To all outcomes: - Mean differences or Standardized Mean Difference and 95% confidence intervals will be calculated for all the variables.	
45.	The statistical model of analysis (*e.g.*	To all outcomes:	

	random or fixed effects model)	- Random effects model -	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I-square.	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Animal species. Gender. Pancreatitis induction method. Natural plant. Dose.	
48.	Any sensitivity analyses you propose to perform	If possible, secondary data analysis according to high/low risk of bias.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	Correction for multiple use of control group.	
50.	The method for assessment of publication bias	Funnel plot, if applicable.	
Final approval by (names, affiliations):			Date: