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	Medicinal plants and Natural Compounds in the treatment of experimental endometriosis: a systematic review	
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5.	Funding sources/sponsors	-Tehran University of Medical Sciences -Iran National Science Foundation -Royan Institute for Reproductive Biomedicine	
6.	Conflicts of interest	The authors report no conflict of interest.	
7.	Date and location of protocol registration	January 29, 2016. SYRCLE	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Systematic search and abstract-screening completed, full text extraction started	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Endometriosis is defined as the extra uterine growth of endometrial tissue, most commonly on the peritoneal and visceral surfaces of the pelvis, and containing both glandular and stromal components. The current treatments are mainly based on inhibiting estrogen and its receptors which are not useful for every patient with endometriosis because estrogen is only one factor in the development of endometriosis. The other treatments also focus on treating the symptoms rather than curing the disease. In addition hormonal treatments interfere with infertility treatments and pregnancy and have different side effects. The recurrence rate is nearly high after both current medical and surgical treatments for endometriosis. In recent years, medicinal herbs and other botanical products have become popular for management of symptoms of several gynaecologic disorders such as endometriosis. The objective of this systematic review and meta-analysis is to provide the preclinical researches on medicinal plants (not Chinese combinations) and its compounds investigated in the treatment of experimental endometriosis. It will also prepare the strengths and limitations of available studies and offer future perspectives in this field.	
	Research question		
11.	Specify the disease/health problem of interest	Endometriosis	
12.	Specify the population/species studied	All animal models used for induction of endometriosis	
13.	Specify the intervention/exposure	Effect of not Chinese medicinal herbs and its compounds on experimental endometriosis	

14.	Specify the control population	Placebo-treated controls	
15.	Specify the outcome measures	Endometriosis regression	
16.	State your research question (based on items 11-15)	1. Compared to placebo or control, is there any treatment based on medicinal plants that is effective in regression of endometriosis? 2. What medicinal plants and secondary metabolites have already been investigated in the treatment of experimental endometriosis? 3. What experimental models are most frequently used to investigate the efficacy of medicinal plants and its compounds in endometriosis?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	☑ MEDLINE via PubMed ☐ Web of Science ☑ SCOPUS ☐ EMBASE ☐ Other, namely ☐ Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	Simplified PubMed search: (Complete search strategy will be defined at final systematic review article) **Medicinal plants or herbal medicine:** Herbs[tiab] OR Herb[tiab] OR Polyphenolic Extract[tiab] OR Phenolic antioxidants[tiab] OR Green tea[tiab] OR Curcumin[tiab] OR Resveratrol[tiab] OR Seed extract[tiab] OR Leaf extract[tiab] OR Fruit extract[tiab] OR Flower extract[tiab] OR Root extract[tiab] OR aqueous extract[tiab] OR methanol extract[tiab] OR ethanol extract[tiab] OR phenol contents[tiab] OR flavonoid contents[tiab] OR botanical extract[tiab] OR Puerarin[tiab] OR Medicinal Plant extract[tiab] OR botanical[tiab] OR dietary supplements[tiab] OR complementary and alternative medicine[tiab] OR CAM[tiab] OR anti-endometriotic agent[tiab] OR Western Herbal Medicine[tiab] OR herbal prescription[tiab] OR herbal extract[tiab] OR herbal medicinal products[tiab] **Endometriosis** ("endometriosis"[MeSH Terms] OR "endometriosis"[All Fields]) OR (endometriotic lesion[tiab] OR endometriotic lesions[tiab] OR endometriosis-like lesions[tiab] OR endometriotic implant[tiab] OR endometriotic implants[tiab] OR endometriosis model[tiab] OR experimental endometriosis[tiab] OR ectopic endometriotic tissues[tiab]) **Animals** Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. Enhancing search efficiency by means of a search filter for finding all studies on animal experimentation in PubMed. Lab Anim. 2010; 44(3):170-5.	

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: Grey literature (Google Scholar)	
20.	Define search strategy for these other sources	Reference lists of included studies and relevant reviews will be checked by two reviewers for additional relevant references not yet identified by our search strategy, based on their title. Possibly relevant references will then be assessed for inclusion as indicated at item 21. Search at Google scholar will be performed by using these key words: (Plant* OR herb*) AND (endometriosis OR endometriotic lesion OR endometriotic lesions OR endometriosis-like lesions OR endometriotic implant OR endometriotic implants OR endometriosis model OR experimental endometriosis) AND animal NOT Chinese	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1)Screening based on title and abstract 2)Screening based on full-text of the eligible articles	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	(a)For each screening phase NS and KK will independently assess eligibility. (b)Disagreements in inclusion will be discussed between the two reviewers until consensus is reach.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	**Inclusion criteria**: Animal studies with control groups **Exclusion criteria**: Non-interventional studies, no control group	
24.	Type of animals/population (*e.g.* age, gender, disease model)	**Inclusion criteria**: All animal models for endometriosis **Exclusion criteria**: In vitro studies or human studies	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	**Inclusion criteria**: Use of herbal or natural compounds for treatment of animal model of endometriosis (no restriction for dosage, timing or frequency) **Exclusion criteria**: Chinese herbal medicine (See Appendix 1) - Combination of different herbal compounds with un known origin- mixture of chemical and herbal treatments- mixture of herbal and other kinds of complementary therapy	
26.	Outcome measures	**Inclusion criteria:** Any outcome related to the severity, progression or reproductive consequences of endometriosis **Exclusion criteria**: no relevant outcomes assessed	
27.	Language restrictions	Inclusion criteria: Only restriction for Chinese language	

		Exclusion criteria: Chinese language	
28.	Publication date restrictions	**Inclusion criteria**: Articles published up to December 2015 **Exclusion criteria**: No past date restriction	
29.	Other	**Inclusion criteria**: Original, full-text publications containing unique data **Exclusion criteria**: Reviews or non-original papers	
30.	Sort and prioritize your exclusion criteria per selection phase	**Selection phase1: (First screening based on title/abstract)** 1. Review papers or non-original papers 2. Not an in vivo animal model 3. Not on disease of interest (Endometriosis) 4. Not about usage of medicinal plants or phytochemicals 5. Combination therapy or use of Chinese herbal medicine 6. Not English **Selection phase: (Second screening based on full text)** 1. Review papers or non-original papers 2. Not an in vivo animal model 3. Not on disease of interest (Endometriosis) 4. Not about usage of medicinal plants or phytochemicals 5. Combination therapy or use of Chinese herbal medicine 6. No relevant outcome measures 7.No appropriate control group 8.Full-text un retrievable	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, title, year of publication, contact author email	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	-Experimental groups -Type of control group (e.g. placebo treatment) -Number of animals in experimental and control groups -Type of randomization	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	-Animal species, Supplier of the animals -Strain -Age -Weight -Endometriosis induction technique (Autologous, homologous, heterologous transplantation by surgery),(injection of endometrial tissues) -Scratching of myometrium layer or not - Type of sham surgery in control group -Estradiol usage during model induction (Dosage, duration, frequency) -With or without ovariectomy -Type of anaesthesia -Number of tissues transplanted in surgery model -Size of transplanted tissues -Transplantation site or location at surgery -Number of surgery -Timing for endometriosis induction before treatment	

34.	Intervention characteristics (*e.g.* intervention, timing, duration)	-Intervention (drug name) -Type of plant extract -Dosage of drug -Duration of treatment -Frequency of drug administration -Route of administration (oral or intraperitoneal injection) -Placebo solution (saline, vehicle, control)	
35.	Outcome measures	Primary outcome: -Size or weight of lesions Secondary outcomes: -Histopathological score of endometriotic lesions -Stress oxidative assessments -Molecular assessments -Immuno-histochemical assessments	
36.	Other (*e.g.* drop-outs)	-Age of sacrificing animals -Anesthetics used for sacrificing -Side effects of drug (weight loss, death, etc.) -Number of animals excluded from statistical analysis -Reason for excluding animals	
	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) NS and KK will independently assess the risk of bias/study quality in each study and (b) Disagreements will be discussed between the two reviewers 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)	**Continuous**: Lesion size (mm), histopathological score, Gene expression **Dichotomous**: incidence of lesion, percentage of positive areas for markers at immunohistochemistry **Counts**: Lesion number If the SD is not presented in the included articles then SD will be estimated from the standard error (SE), 95% CI, the p-value.	

40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1)Extract data from text or tables 2)Extract data from figures 3)Contact authors for data not presented in paper In case of no response within three weeks including a reminder, 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)	Meta-analysis with subgroup analysis and sensitivity analysis for all outcome measures. For outcome measures where a meta-analysis is not possible a qualitative data synthesis of the results from individual studies will be performed.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	If the studies are sufficiently comparable (with regard to design etc.), outcome data will be pooled. Subgroup analyses will only be performed, if the overall meta-analysis contains a minimum of 4 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)	Standardized mean differences (SMD) with 95% Cis will be calculated for outcome measures of continuous and semi-continuous scales for all outcome measures reported as incidences (e.g. number of lesions, we use a risk ratio	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	A random effects model will be conducted as heterogeneity is expected due to differences in animal model, interventions, outcome measures, etc.	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I-squared	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Animal species Endometriosis induction method Medicinal plant Dose of drug Duration of treatment Time between model induction and start of drug Rout of administration	
48.	Any sensitivity analyses you propose to perform	Will be determined (may be subgroup analysis)	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	We need to perform a Holm-Bonferroni correction for testing multiple subgroups. If one or more subgroup analyses cannot be performed due to insufficient data, the p-value will be adjusted accordingly. Also correction for multiple uses of control group will be performed by dividing the number of animals in the control group by the number of comparisons performed with this control group.	

50.	The method for assessment of publication bias	The publication bias will be investigated using a funnel plots and visual analysis of these plots for outcome measures containing 20+ studies. We are aware that funnel plots of SMD are susceptible to distortion and will omit the assessment of publication bias if this is suspected for our dataset. In addition, we aim to perform Egger's test for small study effects for outcome measures containing 20+ studies.	

Final approval by (names, affiliations):
On behalf of all co-authors,
Seyedeh Nargess Sadati, kiandokht Kiani

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