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Catharanthus roseus anti-inflammatory action: A systematic review protocol of *in vivo* and *in vitro* studies

Eka Santi[1], Windy Yuliana Budianto[1*], Widya Nursantari[2], Fatma S Ruffaida[3], Indrya Anggita Sari[4]
[1] Department Pediatric Nursing, Faculty of Medicine, Universitas Lambung Mangkurat, Indonesia
[2] Department of Public Health, Faculty of Medicine, Universitas Lambung Mangkurat, Indonesia
[3] Department of Geriatric Nursing, Faculty of Medicine, Universitas Lambung Mangkurat, Indonesia
[4] Nursing Study Program, Faculty of Medicine, Universitas Lambung Mangkurat, Indonesia

Abstract
Catharanthus roseus is a plant that has been traditionally used for medicinal purposes and belongs to the *Apocynaceae* family. This plant thrives in tropical and subtropical climates all over the world. A variety of bioactive chemicals that have therapeutic benefits can be found in plants. *C. roseus* is known for its significant biological effects, such as antibacterial, antiviral, antifungal, antioxidant, and anticancer properties. The purpose of this systematic review is to meticulously analyze scientific evidence of the anti-inflammatory properties exhibited by *Catharanthus roseus* through *in vivo* and *in vitro* studies. The review aims to answer questions regarding the effectiveness of different plant extracts and their impact on acute inflammation using experimental models. The guidance of Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) will be adhered to in conducting this Systematic Review. Rayyan will be used in the process of selecting evidence and duplicate articles will be removed. Data will be collected using customized data extraction in spreadsheet. the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) will be utilized will be used to assess risk of bias. The results session will present the data synthesis, and some authors may be contacted for unclear or missing data.

Keywords: *Catharanthus roseus,* anti-inflammatory, systematic review, protocol

Introduction

Catharanthus roseus is a plant that has been traditionally used for medicinal purposes and belongs to the *Apocynaceae* family. This plant thrives in tropical and subtropical climates all over the world. A variety of bioactive chemicals that have therapeutic benefits can be found in plants. *C. roseus* is known for its significant biological effects, such as antibacterial, antiviral, antifungal, antioxidant, and anticancer properties [1, 2, 3].

Multiple research papers have shown the healing characteristics attributed to the foundation of *C. roseus* plant, in correlation with the plant's widespread utilization in traditional medicine[4], anti-cancer agent [5], antioxidant [6, 7] as well as anti-inflammatory properties [4].

Numerous studies have been undertaken to validate and establish the biological properties of *C. roseus*. *In vivo* and *in vitro* models have been utilized in some of these studies. In order to streamline research efforts and minimize resource wastage, retrospective and systematic research can aid in outlining the employed methodologies and results, thereby enabling more efficient future research. The purpose of this systematic review is to meticulously analyze scientific evidence of the anti-inflammatory properties exhibited by *Catharanthus roseus* through *in vivo* and *in vitro* studies. The review aims to answer questions regarding the effectiveness of different plant extracts and their impact on acute inflammation using experimental models. Specifically, the review seeks to identify which part of the C. roseus plant and what type of extract exhibit the most compelling evidence of anti-inflammatory effects.

Methods

The guidance of Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) will be adhered to in conducting this Systematic Review [8].

Search Strategy

The search strategy will be designed in consultation with an information specialist.

The search approach will iteratively proceed as follows:

1. An initial limited search of CINAHL. To construct a comprehensive search strategy, we made use of the text words found in the titles and abstracts of relevant publications. In addition to the index keywords that were used to characterize the articles.
2. The search strategy will combine free and controlled terms, including medical subject headings terms. In addition, the terms will also be integrated with Boolean operators.
3. The search strategy, which will be modified to incorporate all the detected keywords and index terms, will be tailored specifically for every database and/or information source that will be utilized. The researcher is going to review:

- Electronic databases using inclusion and exclusion criteria (Scopus, Web of Science, PubMed, CINAHL Complete, and Cochrane Library),
- Search engine (Google Scholar) to find relevant information,

4. To augment the search, both backward and forward citation searches will be conducted to explore further sources.

Study selection

All citations found will be imported to EndNote 21 and then to Rayyan, with the complete references included. Rayyan will be used in the process of selecting evidence and duplicate articles will be removed. The research team will independently screen study titles and abstracts using predetermined inclusion and exclusion criteria. In the next step, two separate reviewers will independently scrutinize the full texts of the selected citations, based on the inclusion and exclusion criteria.

Eligibility criteria

PICOS criteria: 1. Population: Animals (*Rattus novergicus or Mus musculus*) or *in vitro* test; 2. Intervention: Treatment with extracts from different parts of the plant in *in vivo* and/or *in vitro* models; 3. Control: negative (saline or PBS) and positive (standard drug) controls; 4. Outcome: anti-inflammatory action; 5. Study type: experimental studies.

The inclusion criteria are published articles with non-restricted time or language; articles with titles and abstracts accorded to the research questions; *In vivo* and *in vitro* studies, which tested the anti-inflammatory action of *Catharanthus roseus*, regardless of the tested part of the plant and the extract type. In studies, which analyzed other effects, in addition to the anti-inflammatory activity, only such data will be extracted: studies that described mean and standard derivation in tables, graphs, or embedded in the texts.

The exclusion criteria for title-abstract screening were:

1. Studies in human beings, genetic evaluation studies or cancer model studies;
2. Phytochemical studies; morphological and anatomical studies; cytogenetic analysis; ethnobotanical studies;
3. Studies performed in silico or ex vivo models;
4. Treatment with any plant except from the *Catharanthus roseus*;
5. Studies based on interventions with the plant *Catharanthus roseus* in non-inflammatory processes;
6. Animals with previous systematic disease, auto-immune conditions, or any other conditions, which might interfere in the inflammatory model disease evaluated such as obesity, diabetes, or pregnancy;
7. Studies without control group;
8. Toxicity, cell viability outcomes, histological data;
9. Studies without a separated control group or with unavailable data mentioned in the studies.
10. Gray literature.

Data collection process

Data will be collected using customized data extraction in spreadsheet with the following data: First author; Year of publication; Publishing journal; Country of origin/ collection location/ or period of the year; Plant part; Extract type; Extract dose and route of administration; Type of inflammation model or type of assay; *In vivo* or *in vitro* model; Number of animals for group and cell type; Therapeutic scheme; Control used; Evaluated parameters; Results.

The variables analyzed for the two models (*in vivo* and *in vitro*) were plant collection location; plant part; extract type; inflammatory cytokines levels (TNF-α, IL-1); nitrate. Data such as mean, standard deviation and percentage will also be collected.

The variables analyzed for *in vivo* model were: extract dose; route of administration; animal model (rat or mice); the number of animals for group and number of groups; paw edema volume; area under the curve (paw edema); edema ear weight; polymorphonuclear leukocyte count (PMNL); myeloperoxidase levels (MPO); malondialdehyde levels (MDA); glutathione levels; Release of vasoactive amines; peripherical inflammatory pain; plasm leakage; mast cells counting; prostaglandin E2 (PGE2); wound diameter/ ulcerated area.

Risk of bias in individual studies

Two reviewers (FR, WB) will evaluate the risk of bias. To examine the methodological quality, the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) will be utilized. SYRCLE comprises six different types of bias, with ten entries in total. The biases include selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases [9]. Selection bias includes sequence generation, baseline characteristics, and allocation concealment. Performance bias includes random housing and blinding. Detection bias includes random outcome assessment and blinding. Attrition bias includes incomplete outcome data, whereas reporting bias is related to selective outcome reporting. The information regarding bias will be arranged systematically in Rayyan and subsequently exported to a spreadsheet. The reviewers will make judgments and label them as "yes" (low risk of bias), "no" (high risk of bias), or "unclear" (not sufficient information reported).

Synthesis method

Narrative synthesis will be carried out for eligible studies, involving summarization of collected data and descriptive analysis of results. The results session will present the data synthesis, and some authors may be contacted for unclear or missing data.

Apart from using SYRCLE as mentioned above, the quality of evidence will also be analyzed using the indirectness domain, following the GRADE for *in vivo* studies [10]. Additionally, the Grades of Recommendation, Assessment, Development and Evaluation Working Group Guideline Development Tool (GRADEpro GDT) will be utilized [11].

There is no validated checklist to analyze the risk of bias for *in vitro* studies. However, the Science in Risk Assessment and Policy (SCIRAP) tool can be used to assess *in vitro* toxicity studies [12].

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