



Hepatoprotective potential of Ayurvedic drugs: systematic review protocol for experimental studies

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Abstract

To systematically evaluate and provide a critical assessment of the preclinical in vivo and in vitro studies testing the hepatoprotective potential of Ayurvedic medicinal plants and its formulations. This study will be conducted in adherence with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analysis) protocol. A comprehensive search will be performed in databases including PubMed, Scopus, Web of Science, and AYUSH Research Portal from January 2010. In vitro investigations on the hepatoprotective potential of Ayurvedic herbs or drugs will be carried out through established protocols of chemically induced liver injury. The review will compile evidence only from published in vivo and in vitro studies which have tested the hepatoprotective efficacy of Ayurvedic medicinal plants and Classical Ayurvedic formulations. This procedure in detail provides an organised approach for the assessment of the hepatoprotective efficacy of Ayurvedic drugs involving animal studies. Evidence available in this review may assist in adopting scientific evidence-based rational use of certain Ayurvedic drugs in the management of liver diseases, keeping in mind that complete commercial products or toxin-free extract with proper standardisation, toxicity profile in animal models and in humans and clinically established efficient dose form are taken into consideration.

Keywords: Hepatoprotective drugs; Ayurveda; systematic review

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GRADE, Grading of Recommendations Assessment, Development and Evaluation; PRISMA-P, Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols; SOD, superoxide dismutase

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1. Introduction

Liver diseases are among the major public health problems worldwide, leading to about two million deaths per year due to liver cirrhosis and hepatocellular carcinoma (1). Despite advances in the field of modern medicine, controlling liver diseases is a challenging task due to lack of sufficient therapeutic agents, hepatotoxic effects of the synthetic drugs and lack of efficient antifibrotic agents (2). From this viewpoint, the traditional system of medicine, particularly Ayurveda, has attracted attention, as it provides extensive formulations for liver, which are famous for their significant hepatoprotective activities.

The liver ailments have been classified in Ayurveda under the categories of Jaundice (Kamala, Halimaka, Yakrit Vikara) and Anaemia (Pāṇḍu). Ayurvedic literature reported several drugs for the management of liver affections, which include a wide range of herbal and Herbo-mineral drugs, including *Phyllanthus niruri* (Bhūmyaamalākī), *Picrorhiza*

kurroa (Katuki), *Berberis aristata* (Daruharidrā), and compound formulation as *Arogyavardhini Vati* (3, 4). The liver protective action of these medications is assumed to be related to the antioxidant, anti-inflammatory, modulation of liver enzymes, and enhancement in the hepatic regenerative characteristic (5).

The preclinical experimental in vitro and in vivo evidence supporting these statements is described in mechanistic and pharmacological terms. Data from several studies suggest that hepatotoxicity caused by agents like carbon tetrachloride, paracetamol, alcohol, antitubercular drugs, etc., may be ameliorated by Ayurvedic drugs (6). However, owing to differences in study design, drug standardisation, outcome and reporting, there is a need for a methodological synthesis of evidence.

No systematic review has been available to date, which has tried to critically appraise the experimental evidence that has been put forth in support of the hepatoprotective effect of Ayurvedic drugs. An excellent systematic review of

the experimental studies may pave the way for future pharmacological studies, identification of candidates for clinical studies or may facilitate the introduction of Ayurvedic interventions in hepatology based on evidence.

The protocol, therefore, is an attempt to outline a methodology that will be utilised to systematically review the preclinical experimental interventions on the hepatoprotective activity of Ayurvedic drugs. The review will disseminate findings to the research environment, appraise the evidence and identify further need for research.

2. Methodology

2.1 Selection criteria

Types of studies to be included in the review: Both in vitro and in vivo experimental studies that evaluate the hepatoprotective effects of Ayurvedic formulations will be considered.

2.1.1 Inclusion criteria

- Experimental research, including both in vivo and in vitro studies
- Studies featuring a clearly defined control or comparator group
- Investigations assessing the hepatoprotective properties of Ayurvedic formulations or individual drugs
- Published and unpublished studies evaluating Ayurvedic interventions for liver protection, regardless of language
- Only studies published from January 2010 onward will be included

2.1.2 Exclusion criteria

- Non-experimental studies such as observational research, review articles, meta-analyses, case reports, abstracts, letters to the editor, research proposals, and editorial pieces
- Clinical trials or studies exclusively involving human participants
- Studies that do not report clear or measurable outcomes related to hepatoprotective effects
- Research with insufficient or incomplete data, making it difficult to assess the efficacy of the Ayurvedic intervention

Intervention: This review will include Ayurvedic drugs and polyherbal formulations traditionally used for liver protection.

2.2 Control group/ comparator

Hepatocytes treated with known hepatoprotective agents, and hepatocytes exposed to hepatotoxic agents. Animals

receiving only the carrier substance (e.g., saline, DMSO) to ensure effects are due to the Ayurvedic intervention

2.3 Search method for identification of studies

Electronic databases to be searched include the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, EBSCO, AYUSH Research Portal, DHARA (Digital Helpline for Ayurveda Research Articles), IndMED, Clinical-Trials.gov, the World Health Organisation International Clinical Trials Registry Platform (WHO ICTRP), and Shodhganga, a digital repository of Indian theses. In addition, thesis and dissertations available in the public domain, including unpublished postgraduate (P.G.) and doctoral (Ph.D.) work accessible through university and institutional websites, will also be considered from inception to the present.

Backward and forward citation tracking will be performed to identify additional relevant studies. All searches will be updated prior to the final analysis. In cases where results for registered trials are not published, the principal investigators will be contacted via email to obtain the necessary data. The search window will cover studies published from January 2010 to the present, and no language restrictions will be applied during the selection process.

2.4 Main outcome(s)

Assessment of liver function biomarkers such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Bilirubin.

2.5 Additional outcome(s)

Histopathological analysis of liver tissues, Cytotoxicity assays, Gene and protein assays related to liver pathology such as inflammatory markers, oxidative stress markers such as malondialdehyde (SOD) and superoxide dismutase.

2.6 Data collection

2.7 Data extraction

Two review authors (HP and SS) will screen titles and abstracts of all identified articles independently. According to the Preferred Reporting Items for systematic reviews and meta-analysis (PRISMA) recommendations, the author name and publication year, study setting, animal model, method, the details for the intervention and comparators, outcome indicators, and result data will be extracted using a standardised data collection form. Regarding the characteristics of the included studies, the species and strain of the animal will be recorded, as well as the age and the initial mean body weight at the beginning of the study. Disagreements at the screening or extracting stage will be solved by consensus, or a fourth reviewer (PK) if required.

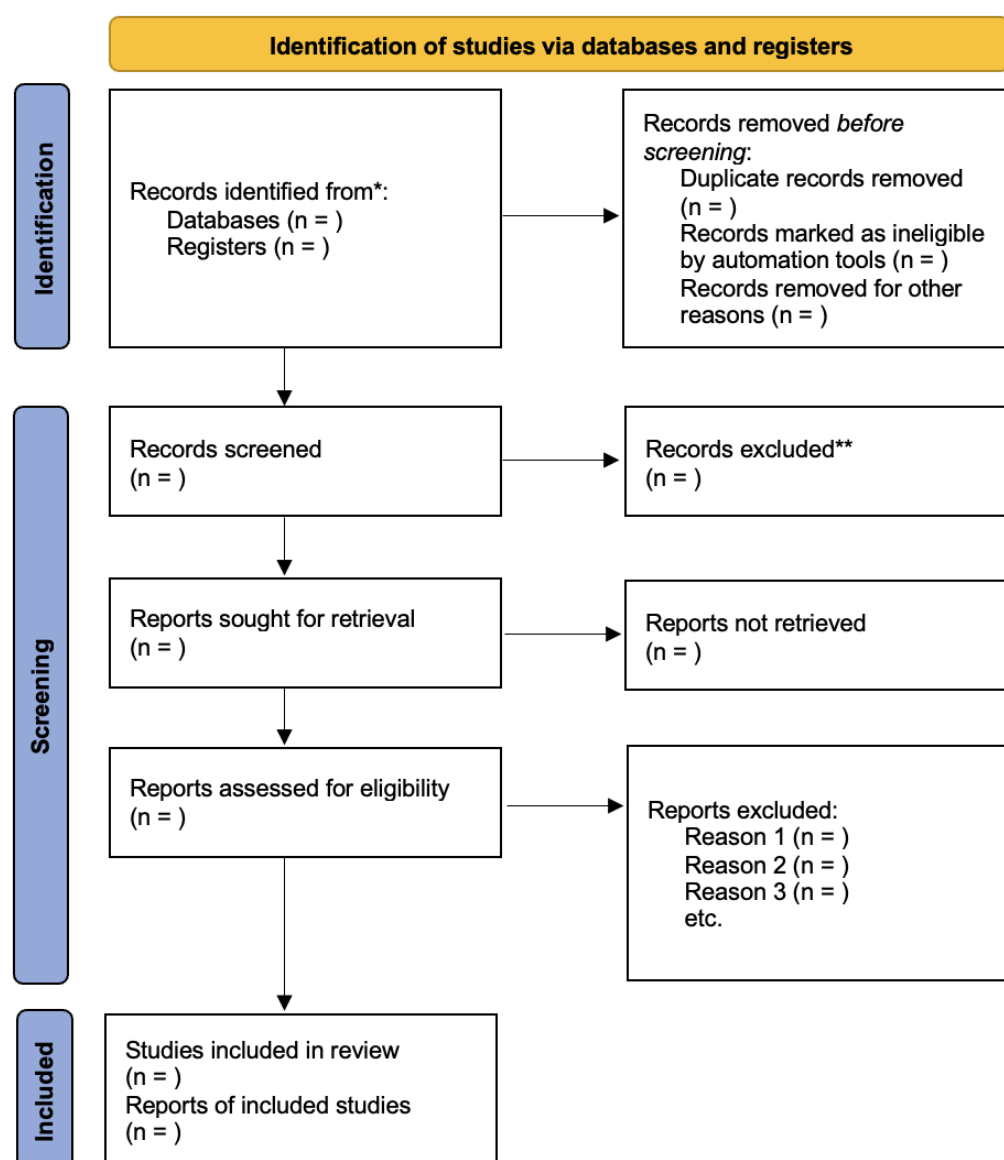


Figure 1: Flow chart showing the review process. Counts should be completed when the systematic review is conducted.

2.8 Data synthesis

Quantitative data of primary (liver function biomarkers, i.e., ALT, AST, ALP and bilirubin) and secondary outcomes (histopathological score, cell viability assay, mRNA/protein expression levels of inflammation and oxidative stress mediators, i.e., MDA and SOD) will be presented in the form of tables. For all included studies, descriptive synthesis will be taken place that includes the study design, animal/cell models, Ayurvedic drug/formulation involved, dosage, duration of administration, type of hepatotoxic challenge. The narrative synthesis will summarise the alterations in liver biomarkers, tissue findings, and molecular results, presenting differences between treated and control groups. If the heterogeneity is acceptable between studies regarding the outcome measures and experimental models, a meta-

analysis will be performed using the fixed-effects model. Effects will be estimated with SMD or MD for continuous outcomes, including 95% CIs.

RevMan software will be used for data synthesis. RevMan is well-known and very easy-to-use software for systematic reviews and meta-analyses.

2.9 Assessing the heterogeneity

Heterogeneity between studies will be measured by the I^2 statistic, and heterogeneity factors (i.e., type of Ayurvedic drug, model, or study quality) will be evaluated by subgroup analysis or meta-regression, if applicable.

2.10 Subgroup analysis

Subgroup analysis will be performed to explore potential sources of heterogeneity and the effects of treatment and to assess the variation in studies with different Ayurvedic type of formulations, study design, method of liver damage induction used (drug used), species of animals used, dose, route of administration and duration of treatment.

2.11 Risk of bias

The risk of bias will be evaluated by SYRCLE's risk of bias tool and CAMARADES checklist for study quality. SYRCLE's risk of bias tool is specifically tailored from the Cochrane Risk of Bias tool for application in animal intervention studies. Selection bias, detection bias, attrition bias, reporting bias and performance bias were assessed. Risk of bias tools will be unavailable for in-vitro studies, as there are no available validation tools. Risk of bias assessment Three review authors (HP, SS and AAT) will assess the risk of bias of each included trial independently. Any disagreements will be resolved by consensus or by consulting a fourth reviewer (PK).

To reduce potential for publication bias, articles will be included regardless of language and studies published and unpublished. The quality of evidence across studies will be assessed according to the GRADE criteria. This method evaluates the confidence in the evidence across a number of domains, including risk of bias, addressing the methodological quality and biases in the included studies; inconsistency, considering unexplained heterogeneity in the results of the included studies; indirectness, related to the generalizability of the evidence to the question of interest; and imprecision, which considers the precision of effect estimates among the included studies.

2.12 Data analysis

Three review authors (HP, SS and AAT) will independently evaluate evidence quality for each outcome. The results will be summarized with a table. When the studies are quite homogeneous with respect to design, population, interventions, or outcome measurements and at least five studies have reported similar data for the same outcome, a meta analysis will be performed. Heterogeneity will be determined with the I^2 statistic. If fewer than five studies are found for a specific outcome or if there is substantial heterogeneity in measurements of the outcome between studies, a narrative synthesis will be undertaken.

Descriptive analysis: The liver function biomarkers including ALT, AST, ALP, Bilirubin will be reported and described using descriptive statistics for the overall outcomes (main outcomes) for each study. This will involve computing the mean, median and standard deviation of baseline and post-intervention levels of biomarkers. For other endpoints such as histopathologic examination, cytotoxicity examinations, gene/protein expression in relations with liver pathogenetic mechanisms (e.g., inflammatory markers, oxidative

stress markers), reporting statistics will provide descriptive statistics such as mean, median, and standard deviation where appropriate. The expression values (e.g., percent change) will be included if provided to capture the impact of the Ayurvedic interventions.

3. Discussion

This systematic review protocol describes the methods of evidence synthesis, which are structured and repeatable, adopted to summarise pre-clinical evidence on hepatoprotective effect of Ayurvedic drugs in in-vivo and in-vitro experimental models. Numerous herbal and herbo-mineral formulations have been described for liver disorders in ancient Ayurvedic literatures; however, a meticulous review of experimental data in modern biomedical journals is still lacking.

With the current worldwide rising prevalence of liver diseases, which encompasses drug-induced hepatic injury, NAFLD and viral hepatitis, there is a need for the availability of safe, efficacious and economically feasible hepatoprotective agents. Anecdotal claims are made in uncontrolled studies for the efficacy of Ayurvedic preparations to treat this condition, many of them tested over centuries to the satisfaction of practitioners; some, and for other conditions, have well-known properties (such as antioxidant action, hepatocellular membrane stabilisation, anti-inflammatory properties and effects on detoxification enzymes). However, lacking a structured appraisal of the included studies, we cannot recommend their evidence-based incorporation into contemporary practice.

This will add to the systematic identification, appraisal and synthesis of the literature in preclinical data and in addressing this gap at the preclinical level, this review aims to help fulfil this need. Both animal (in vivo) and cell line (in vitro) models will be incorporated and supplemented with use of the review to provide a full picture of the mechanistic and functional consequences of hepatoprotection according to experimental platforms. The reviewing also intended to assess the methodological quality, standardisation of extracts, dosage, outcome measures and other constraints in the included studies.

Information from data analysis should indicate a promising research topic and research gap with a focus on extract standardisation, toxicity testing and reproducibility. This news piece might influence future experimental design and translational and clinical understanding. In addition, such a review could be helpful to identify specific interventions from Ayurveda that require translation in HA clinical practice; scientific necessity for regulatory scrutiny and use of pharmacovigilance.

In order to be transparent and prevent bias, we will register this protocol following the PRISMA-P guideline. The findings of this systematic review are expected to contribute to the functional blending of traditional wisdom and modern advances in hepatology and integrative medicine and

for the practical applications regarding the management of liver disorders.

4. Conclusion

This systematic review protocol provides a structured and reproducible framework for critically evaluating the preclinical evidence on the hepatoprotective potential of Ayurvedic drugs and formulations in both in vivo and in vitro experimental models. By systematically identifying, appraising and synthesising evidence on liver function biomarkers, histopathological changes, cytotoxicity, inflammatory and oxidative stress markers, the review is expected to clarify the current strength and limitations of the available evidence. Particular attention to methodological quality, risk of bias, standardisation of interventions, dosage, experimental models and outcome measures may help identify sources of heterogeneity and important research gaps. The findings may also help identify promising Ayurvedic interventions for further pharmacological and clinical investigation. However, preclinical evidence alone will not establish clinical efficacy or safety; appropriately standardised preparations, toxicity assessment, reproducible experimental methods and well-designed clinical studies will be essential before translation into routine clinical practice. Overall, this review is intended to strengthen the evidence base for Ayurvedic hepatoprotective interventions and support the responsible integration of traditional Ayurvedic knowledge with contemporary evidence-based hepatology.

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Authors' contributions

All authors contributed equally and approved the final version.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical issues

None.

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