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Nature and mechanism of immune boosting by Ayurvedic medicine: A systematic review of randomized controlled trials

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Abstract

BACKGROUND

Many Ayurvedic preparations are claimed to have immune-boosting properties.

AIM

To compile evidence on the nature and mechanism of immune system enhancement by Ayurvedic preparations in both healthy and sick individuals, from published randomized clinical trials (RCTs).

METHODS

After prospectively registering study protocol with PROSPERO, we searched PubMed, DOAJ, Google Scholar, three dedicated Ayurveda research portals, two speciality Ayurveda journals, and reference lists for relevant records published until 02-06-2021 using appropriate search strategies. Baseline features and data pertaining to the nature and mechanism of immune system function were extracted from all eligible records. Methodological quality was assessed using the Cochrane RoB-2 tool.

RESULTS

Of 12,554 articles screened, 19 studies reporting 20 RCTs (17 parallel group design, 3 crossover design) with 1,661 unique patients were included; 11/19 studies had Indian

first authors. Healthy population was included in 9 studies of which 1 study included pregnant women and 2 included paediatric population; remaining studies included patients with different health conditions including 1 study with COVID-19 patients. A total of 21 Ayurvedic interventions were studied, out of which 5 were composite mixtures. The predominant route of administration was oral; dose and frequency of administration of the intervention varied across the studies. The results reported with 5 RCTs exploring 5 Ayurvedic interventions were incomplete, ambiguous, or confusing. Of the remaining 16 interventions, indirect evidence of immune enhancement was reported with 4 interventions, while lack of the same was reported with two interventions. Enhancement of T helper cells and NK cells was reported with 3 and 4 interventions, respectively, while the pooled results did not clearly point toward enhancement of other components of the immune system, including cytotoxic T cells, B lymphocytes, immunoglobulins, cytokines, complement components, leucocyte counts, and other components. Nine of the 20 RCTs had a high risk of bias, and the remaining 11 RCTs had some concerns according to RoB-2.

CONCLUSION

Various Ayurvedic preparations appear to enhance the immune system. Enhancements in NK cells and T helper cells could contribute, while the role of other components of the immune system is unclear.

INTRODUCTION

As of September 2021, the ongoing COVID-19 pandemic has seen at least two waves in almost all regions of the world, including India, with many predictions hinting at a global third wave due to new SARS-CoV-2 variants.^[1] The remarkable trail of destruction left by the pandemic, coupled with the non-availability of a drug-of-choice to treat the condition,^[2] has resulted in an almost complete dependence on the development of safe and efficacious vaccines that can protect individuals against

existing and potential virus variants. Despite developments in vaccinology, it is common knowledge that a complete protection against all future variants of the virus cannot be absolutely guaranteed by even the most advanced vaccines. At the same time, non-neutralizing antibodies that cause antibody-dependent enhancement (ADE) of the immune system are reported to paradoxically exacerbate SARS-CoV-2 infection, leading to worsened organ damage.^[3] This has led to the realization that therapies that produce a sufficiently strong, but not hyperactivated, immune system can lead to a healthier society and can contribute significantly to the fight of mankind against not only the COVID-19 pandemic but also future healthcare challenges of infectious disease origin. Consequently, healthcare systems in many parts of the world, especially India, have turned their attention toward potential therapies that can produce a non-specific and unaided enhancement of the immune system, as a complementary step to vaccine development.^[4,5]

Ayurveda is the Indian traditional system of medicine, and has a history since the 2nd century BC. The core pathophysiological principles of Ayurveda surround the concept of loss of balance with respect to the three humours (*Tridoshas*), five elements (*Pancha mahabhootas*), and seven tissues (*Saptadhatu*s) of the human body. Various Ayurvedic treatments are described for diseases affecting different systems of the body, and most Ayurvedic medicinal items are sourced from natural ingredients. Classical texts of Ayurveda also have mentions about management of epidemics,^[6,7] and has defined 'immunity (*Bala*)' as the 'ability of the body to prevent and arrest the progression of disease for maintaining homeostasis'.^[4] Few facts – such as, the classification of immune system in Ayurveda into *Sahaja* (innate), *Kalaja* (chronobiologic), and *Yuktikrut* (acquired) closely resembling the modern medical classification of immune system,^[4] and Ayurvedic immune-boosting preparations such as *cyavanaprasa* having stood the test of time – are testaments to the near-accurate understanding of our body's immune system by the ancient Ayurveda pioneers. Thus, it is not surprising that the Ministry of AYUSH (Ayurveda, Yoga, Unani, Siddha, and Homoeopathy) of the Indian Government released an advisory recommending various

natural therapies to develop immunity against COVID-19.^[8] Many components named in the said recommendation have been reported to produce immune enhancement through modulation of multiple immune system pathways,^[8] and also through psychoneuroimmunological mechanisms.^[5] However, since most of these observations are through pre-clinical and non-human studies, we were interested to know if these pre-clinical observations hold good when the Ayurvedic preparations are studied after human administration.

With this background, we performed the present systematic literature review (SLR) with an objective for gathering evidence towards the nature and mechanism of enhancement of human immune system by the administration of Ayurvedic preparations, from published randomized clinical trials (RCTs). The research question that we were looking forward to find an answer through this SLR was: ‘what is known from published RCTs about the nature and mechanism of the impact of consuming ayurvedic medicine on enhancing the immune system activity of healthy or sick humans?’

MATERIALS AND METHODS

2.1. Study Protocol

The SLR protocol was drafted following the guidelines given in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, and was subsequently refined through internal discussion. The final version of the protocol was prospectively registered with PROSPERO on 10th July 2020, with ID CRD42020191289.

2.2. Eligibility Criteria

To identify potentially relevant articles that answered our research question, the following eligibility criteria were drafted: ‘Population’ was all studies involving healthy or sick adults with any form of illness (acute or chronic). The protocol was subsequently amended to include humans of all age groups, including paediatric and geriatric individuals. We did not restrict the population with respect to the age or gender of the

individuals, presence of any form of disease, presence of comorbidities, or presence of special situations such as pregnancy. 'Intervention' included any type of Ayurvedic medicine, given as a combination or single drug. Studies that did not explicitly mention whether the intervention was an 'Ayurvedic medicine' or not were included in the review, if the intervention was found in the work 'Indian Medicinal Plants: an Illustrated Dictionary' by Khare CP.^[9] We also used the same resource to compile the Ayurvedic name of various interventions. Papers that included interventions only belonging to other systems of medicine (including modern/ Western medicine, traditional medicines of other regions/ countries, and other systems of medicine including Unani, Siddha, Homoeopathy, Yoga, Naturopathy, Osteopathy, and Chiropractic) were excluded. We did not restrict studies based on the 'Comparator', and included all studies regardless of whether or not there was a comparator. 'Outcomes' of interest included any description of enhancement, augmentation, stimulation, increasing, or strengthening of the immune system, either directly (through observation on the effect of component/s of the immune system) or indirectly (through observation of effect of said enhancement of immune system, by means of surrogate markers such as relief from illness, quickness of recovery etc). Studies that described immunomodulation instead of immune enhancement, and studies that did not describe any immune system effect, were excluded. 'Study design' included only RCTs. Papers not describing primary data (such as narrative or systematic reviews, letters to editors, opinion pieces, commentaries, editorials, brief communications, news items, *etc.*), case studies, case reports, and studies describing non-human experiments (including in-vitro studies, in-vivo studies, in-silico experimentation *etc.*), and studies not in English language were excluded from our review.

2.3. Literature Search, Data Extraction, and Quality Assessment

Following the eligibility criteria described above, a PubMed literature search strategy was drafted and modified through internal discussion. Using the refined search strategy, a systematic literature search was performed in PubMed/MEDLINE, from their inception till 2nd June 2021. Recognizing that many research articles on Ayurveda

are published in journals not indexed in PubMed, and following the recommendations by Aggithaya *et al* (2015),^[10] we performed an additional literature search in Directory of Open Access Journals (DOAJ),^[11] AYUSH research portal,^[12] Digital Helpline for Ayurveda Research Articles (DHARA),^[13] Annotated Bibliography of Indian Medicine (ABIM),^[14] and Google Scholar. Furthermore, we also performed targeted journal search in two Ayurveda speciality journals (Ayu, and Journal of Ayurveda and Integrative Medicine), using a combination of search terms and Boolean operators such as “Ayurveda”, “Ayurvedic medicine”, “Immune”, “Immune stimulating”, “Immune enhancing”, “Immunomodulatory”, and “Randomized clinical trials”. The detailed PubMed search strategy is presented as **supplementary table 1**, and a brief account of searches performed in each of the portals mentioned above is provided as **supplementary table 2**. Finally, we also scanned reference lists of relevant studies to identify potentially eligible records that were missed through database search.

After pooling all the eligible records, we identified the most eligible articles for our review, and extracted relevant data from these papers after thoroughly studying the full texts of each article. Extracted data, such as data related to the study details (year of publication, country of the first author, study design, details of the intervention and comparator), participant details (participant profile, number, age and sex), details of the intervention and comparator (Ayurvedic, commercial, and scientific names, composition, dose, frequency, duration, and route of administration), and outcome details in terms of improvement from baseline, were entered into a predefined data entry grid. All outcomes related to the immune system (or *Ojas*) were considered for this study, and included direct variables (such as leukocyte subtypes including T lymphocytes, B lymphocytes, NK cells, and various types of myelocytes; immunoglobulins, complement components, and cytokines) and indirect variables (such as absenteeism, number of healthy days and sick days, number of doctor visits, number of symptoms, *Ojas* score, etc). The methodological quality of the included studies was assessed using the Cochrane RoB-2 tool.^[15] We used the official Microsoft

Excel tool provided by the Cochrane Foundation for implementing RoB-2, and we used separate tools for parallel group RCTs and crossover RCTs.^[16]

Subsequent to executing the literature search, two authors [BNV, AD] independently screened the pooled articles for their inclusion in the study, extracted data, and assessed the articles for risk of bias; any disagreements were resolved through discussion and reconciliation that was moderated by another author [DD].

2.4. Statistical Analysis and Data Availability

Study selection and data extraction were done electronically in Microsoft Excel. To assess the inter-rater reliability (IRR) of the study inclusion and the methodological quality assessment of the included articles, Cohen's kappa value was calculated using SPSS version 20. The cut-off points for the kappa statistic were interpreted as ≤ 0.20 = slight agreement; 0.21–0.40 = fair agreement; 0.41–0.60 = moderate agreement; 0.61–0.80 = substantial agreement; 0.81–0.99 = near-perfect agreement; and 1.00 = perfect agreement.^[17] Statistical review of the study was performed by an author who is a biomedical statistician [BNV]. All datasets used to derive conclusions in this study are available with the corresponding author on reasonable request.

RESULTS

3.1. Study Selection, Baseline Characteristics

From an initial pool of 12,554 potentially eligible records, ¹19 studies were included for data extraction and review. **Figure 1** depicts the study selection process.

The 19 included studies were published from 2005 to 2021. A total of 20 RCTs were reported in the 19 included papers: 16 articles described parallel group RCTs, two described crossover trials, and one article reported two RCTs: one parallel group RCT, and one crossover RCT. The first authors of the 19 papers were from 6 different countries, with India being the most frequent country of affiliation (11 studies), followed by Japan and USA (2 studies each); there was one study each with first authors from Germany, Iran, Romania, and Turkey. The participant profile ranged from healthy participants (6 studies, healthy adults; 2 studies, healthy pediatric population; 1 study,

healthy pregnant women), patients with cancer (3 studies, gastrointestinal cancer; 1 study, head and neck cancer), patients with diabetes and HIV (2 studies each), and patients with COVID-19 and allergic rhinitis (1 study each). Among the 17 studies that included adult participants, two included only females, one study included only males, and the remaining 14 studies included participants of either gender. The sample size of the studies ranged from 5 to 627, with a total of 1,661 unique participants across the 19 articles. The complete baseline characteristics of the included studies are summarized in **table 1**.

Two of the included studies explored two interventions, and the remaining 17 studies explored one intervention each, leading to a total of 21 interventions. The predominant route of administration of the intervention was oral ($n = 17$), with one study each delivering the intervention through subcutaneous injection, intravenous infusion, and oil dripping on the forehead; details of route of administration of the intervention were missing in one study. Among the 17 orally administered interventions, the formulations were capsules in 5 studies, and *rasayana* in 3 studies; one study each used other formulations including tablet, tea, *cyavanaprasa*, *kasaya*, *bhasma*, *ghrita*, *churna/ kalka*, and tincture, and one study did not provide information about formulation. Details of dosing and duration of administration of the intervention varied across the studies. Placebo was used as the comparator in 6 studies. The complete details of the interventions and comparators among the included studies are summarized in **table 2**. The detailed composition of the different composite preparations used as interventions in 5 of the studies is provided as **supplementary table 3**.

3.2. Main Findings

Of the 19 included studies, 15 studies provided information about the impact of the intervention on at least one component of the immune system (direct evidence), 3 studies provided information about the impact of the intervention on the overall health status of the participant (indirect evidence), and one study provided both direct and indirect evidence. The description of the results was not uniform across various studies,

with different units of measurement used to express similar outcomes; this prevented us from performing a meta-analysis of the results.

3.2.1. Immune Enhancement: Direct Evidence

3.2.1.1. Impact on T Lymphocyte Subsets (Excluding NK Cells)

CD4 Lymphocyte counts were found to be significantly enhanced after perioperative mistletoe administration for 14 days among patients undergoing surgery for GI cancer.^[18] Among patients with HIV, CD4 Lymphocyte counts were found to be enhanced after a 90-day consumption of *Shilajatu Rasayana*^[19] and *Ranahamsa Rasayana*,^[20] as compared to standard care. However, the increase in CD4 Lymphocyte count caused by the consumption of aged garlic extract for 12 wk was not statistically significant among patients with GI cancer, and was less than that observed with matching placebo.^[21] The results reported in three papers were ambiguous: although *Glycyrrhizia glabra* tincture increased CD4 Lymphocytes by 8.26% in 24 h in healthy individuals, the statistical significance of this finding was not reported, and the sample size was only five patients.^[22] Significant enhancement of CD4 Lymphocyte count was also not observed with *Nigella sativa* seed supplementation, and the data was not presented with clarity.^[23] *Tulsi* was reported to significantly increase CD4 Lymphocytes in 4 wk compared to placebo among healthy individuals, but this article did not provide any numbers to support this claim.^[24]

CD8 Lymphocyte counts were significantly decreased with perioperative mistletoe administration for 14 days.^[18] Aged garlic extract brought about a non-significant increase in CD8 Lymphocyte count over 12 wk, which was also seen with matching placebo.^[21] The remaining three papers presented results with less clarity: though *Glycyrrhizia glabra* tincture increased CD8 Lymphocyte by 2.89% in 24 h in healthy individuals, the statistical significance of this finding was not reported, and the sample size was only five patients.^[22] Significant CD8 Lymphocyte count enhancement was observed with *Nigella sativa* seed supplementation among allergic rhinitis patients, but the presented results lacked clarity.^[23] *Tulsi* did not have significant effect on CD8

Lymphocyte counts among healthy individuals, but this paper did not provide any numbers to back this claim.^[24]

The impact of Ayurvedic preparations on other types/ subtypes of T cells was presented in four papers. Perioperative mistletoe administration was associated with a significant increase in T lymphocyte count, CD3 Lymphocyte count, and CD4/ CD8 ratio among patients with GI cancer,^[18] and *Varunadi Ghrita* consumption for 1 year was found to significantly increase CD3 Lymphocyte count among patients with head and neck cancer after complete remission.^[25] Although aged garlic extract consumption for 45 days was reported to have a significantly higher $\gamma\delta$ -T cell proliferation index among healthy adults compared to placebo, numbers to support this claim were not reported in this paper.^[26] Finally, *Nigella sativa* combined with specific immunotherapy was not found to significantly alter CD3 Lymphocytes among patients with allergic rhinitis, but this article did not present the results with clarity.^[23]

3.2.1.2. Impact on NK Cell Count and NK Cell Activity

NK cell counts were found to be significantly enhanced by perioperative administration of mistletoe for 14 days among patients undergoing surgery for GI cancer,^[18] and also by consumption of aged garlic extract capsules for 12 wk among patients with inoperable gastrointestinal cancer.^[21] Administration of *Varunadi Ghrita* for 1 year was associated with a marginal but significant increase in NK cell counts among patients with head and neck cancer.^[25] *Tulsi* produced a significant increase in NK cell activity over 4 wk among healthy volunteers, but this article did not present the complete results to strengthen its claims.^[24] No significant changes in NK cell counts were observed with either *Glycyrrhizia glabra* consumption^[22] or *Nigella sativa* seed supplementation,^[23] but results presented in both these articles were incomplete.

NK cell activity was found to be significantly enhanced after 7 days of surgery with a single-dose mistletoe extract IV infusion perioperatively among patients undergoing colorectal carcinoma resection, compared to standard care.^[27] Among patients with inoperable GI cancer, aged garlic extract administered for 12 wk was associated with a significant increase in mean NK cell activity %, but a non-significant

reduction in mean NK cell activity per 100 cells.^[21] Regular consumption of tea fortified with several Ayurvedic herbs over 2 mo by healthy individuals was associated with a significant enhancement in NK cell activity compared to regular tea; however, the crossover design of the trial did not have a sufficient wash-out period, and there were inconsistencies with the numbers in the results.^[28] Next, among healthy females, a 30-minute *Shirodhara* treatment with sesame oil was associated with a significant enhancement in NK cell activity compared to the control supine position for 30 minutes,^[29] and among healthy volunteers, the consumption of aged garlic extract for 45 days was associated with a significant enhancement of activated state of NK cells as well as NK cell proliferation index compared to placebo,^[26] however, both these papers did not provide numbers to fully substantiate these claims.

3.2.1.3. Impact on B Lymphocytes and Immunoglobulins

While B lymphocyte counts were significantly enhanced by the consumption of *Varunadi Ghrita* for 1 year among patients with head and neck cancer remission,^[25] a significant change was not reported with consumption of mistletoe extract,^[18] *Glycyrrhizia*,^[22] *Nigella*,^[23] and *Tulsi*.^[24] It is however worthwhile to note that the last 3 studies had methodological or reporting issues. Next, perioperative consumption of Mistletoe extract was associated with a significant increase in serum levels of IgA and IgM, and also of IgG (significance not mentioned);^[18] however, a significant impact was not found on serum levels of IgG, IgM and IgA by saffron consumption,^[30] on serum IgG levels of infants by calcined gold powder consumption,^[31] or on cord blood IgG levels by consumption of *Rasayana Avaleha* by the pregnant mother.^[32] Interestingly, the last study concluded that *Rasayana Avaleha* enhanced foetal immunity level, despite absence of strong evidence pointing towards the same.

3.2.1.4. Impact on Complement Components and Cytokines

While serum levels of complement C3 and C4 proteins were found to significantly increase after 14 days of perioperative administration of mistletoe extract among patients undergoing surgery for GI cancer,^[18] no significant changes in the serum levels of these proteins were observed after daily consumption of saffron tablets

for 6 wk among healthy individuals.^[30] Among healthy adult volunteers, the consumption of aged garlic extract for 45 days was associated with non-significantly reduced serum levels of TNF- α and IFN- γ levels compared to placebo; this 'decrease in cytokine levels' was interpreted by the authors to suggest that consumption of aged garlic extract resulted in 'enhancement of the immune system' in the sense that, after the consumption of aged garlic extract, 'eradication of pathogens causing flu-like illnesses' could be achieved by lower levels of these cytokines.^[26] By contrast, consumption of *Tulsi* capsules for 4 wk was found to significantly increase the levels of IFN- γ and IL-4 in healthy adult volunteers, and this 'increase in cytokine levels' was also interpreted as supporting the observation that *Tulsi* resulted in mounting 'an effective immune response'.^[24] The latter paper also did not provide complete numbers to substantiate the claims.

3.2.1.5. Impact on WBC Counts and Granulocytes

Even though total WBC counts were enhanced by perioperative mistletoe administration after 14 days, the enhancement seen with standard care was found to be numerically higher.^[18] Total WBC counts were not found to be significantly enhanced by administration of aged garlic extract,^[21] saffron,^[30] and calcined gold powder.^[31]

Total lymphocyte counts were significantly enhanced by perioperative mistletoe administration among patients with GI cancer.^[18] While calcined gold powder was found to significantly enhance lymphocyte counts among infants < 1 mo but not in older infants, this paper did not provide numbers to back their claims.^[31] Saffron was not associated with a significant change in total lymphocyte counts as well.^[30]

While significant changes in absolute neutrophil counts were not reported with the use of saffron^[30] and calcined gold powder,^[31] absolute neutrophil counts were significantly reduced by administration of *Ranahamsa Rasayana* for 90 days among patients with HIV.^[20] Phagocytic function by neutrophils was found to be significantly enhanced after a 1-month treatment with *Tinospora cordifolia* (among patients with diabetic foot ulcer)^[33] and *Nigella sativa* (among patients with allergic rhinitis).^[23]

No significant alterations in eosinophil or basophil levels were reported with saffron administration.^[30] Absolute eosinophil count was found to significantly decrease after a 4-week administration of calcined gold powder among infants aged < 1 mo but not among older infants; there were no significant changes in monocyte counts in either age group. However, this paper did not provide complete results to strengthen these claims.^[31]

3.2.1.6. Impact on Other Immune System Variables

Perioperative subcutaneous mistletoe administration over 14 days was found to significantly increase the counts of CD2 Lymphocytes (comprising of T lymphocytes and NK cells) after 14 days among GI cancer patients undergoing surgery.^[18] Perioperative intravenous single-dose mistletoe infusion among GI cancer patients undergoing surgery, was associated with a significant lowering of HLA-DR antigen expression, which was also seen among patients who received standard care.^[27]

3.2.2. Immune Enhancement: Indirect Evidence

Four studies reported indirect evidence of immune system enhancement following various Ayurvedic treatments.

Healthy adults who consumed capsules containing aged garlic extract for 90 days were found to have a reduced severity of cold and flu in terms of a significantly lower number of symptoms, significantly lower decrease in activity and days of reduced activity due to illness, and significantly lower number of work days missed due to illness, compared to volunteers taking matched placebo capsules. However, a significant difference between these two groups was not found with respect to illness incidence, number of days with symptoms, number of symptoms per illness, and number of doctor visits. These observations were attributed to the enhancement of activities of NK cells and $\gamma\delta$ -T cells by aged garlic extract.^[26]

Consumption of *Cyavanaprasa* with milk by healthy children aged 5-12 years for 6 mo was found to be associated with improved health compared to control, in terms of significantly lower number of episodes of infection/ allergy (overall number of episodes, and episodes of mild and moderate severity), significantly shorter duration of

illness (overall duration of illness, and illnesses of mild and moderate severity), significantly more children without infection/ allergy, and significantly lower absenteeism due to illness, both in terms of children reporting absenteeism as well as the number of days of absenteeism due to illness. Other findings not reaching statistical significance included a lower number and shorter duration of severe episodes of infection/ allergy, and a lower number of children with infection/ allergy.^[34]

The 'Ojas score', which signified immune function, was found to be significantly improved after 3 mo of administration of both the Ayurvedic interventions studied (*Mamajjaka* and *Shilajatu*) as well as the control treatment, and the improvement caused by both the Ayurvedic interventions was found to be significantly higher than that seen with the control treatment.^[35] Finally, the number of COVID-19 patients with positive RTPCR results was zero, after 10 days of treatment with either *Vyagradhi Kashaya*, or ginger-garlic, or Vitamin C.^[36]

The complete details of the main findings of all the included studies are summarised in **table 3**.

3.2.3. Safety

Nine of the 19 included studies did not report the safety profile of the Ayurvedic intervention, and eight studies reported not finding any new safety signals of concern. Rais *et al* reported that 2/40 patients receiving *Vyagradhi Kashaya* developed loose stools and subsequently discontinued the treatment, and 3/40 patients receiving ginger-garlic developed burning sensation in the abdomen and were conservatively managed.^[36] After receiving *Ranahamsa Rasayana*, 2 patients were reported to have a mild burning sensation over the body, transient mouth ulceration, and mild drowsiness, which led to discontinuation of treatment.^[20]

3.3. Risk of Bias Assessment of Included Studies

As per RoB-2 tool, Nine of the 20 RCTs described in the 19 included studies had high risk of bias, and the remaining 11 RCTs had some concerns of bias. Only 3 RCTs gave adequate information about randomization process; most of the RCTs used standard methods to measure the outcomes, leading to domain 4 having the highest

number of studies with low risk of bias. Since none of the studies provided information about pre-specified statistical analysis plan, all 20 RCTs received a 'some concerns' rating for domain 5, leading to a similar rating for overall risk of bias. The risk of bias summary is presented in figure 2 and table 1, and the complete analysis of risk of bias assessment is available as supplementary table 4.

3.4. Inter-rater Reliability

The IRR for study selection between the two reviewers was substantial, with Cohen's kappa value being 0.743 (95%CI 0.714-0.772) and 0.681 (95%CI 0.652-0.710) for title-abstract screening and full-text screening, respectively. For the methodological quality assessment, an agreement between the two reviewers with respect to the methodological quality was achieved with 16/20 RCTs, with 4 papers needing mediation, leading to an inter-rater agreement of 84.21%. The Cohen's kappa value for risk of bias assessment was 0.636 (95%CI 0.607-0.665), indicating substantial IRR.

DISCUSSION

The most prominent finding of our study is that while there is reasonably significant indirect evidence of immune system enhancement by different Ayurvedic interventions, especially *cyavanaprasa*, the same cannot be said about direct evidence. In other words, we can say with some confidence that some Ayurvedic medicines have been shown to reduce the duration of illness and improve overall good health after long-term consumption, indirectly indicating that the immune system might have been boosted; however, the exact mechanism by which these effects occur is not yet clear. By looking at the summary of evidence in our review, it appears that enhancement of number and activity of NK cells and T helper cells might be responsible for such an enhancement, while the role of other components of immune system, including cytotoxic T cells, B lymphocytes, immunoglobulins, complement components, cytokines, and WBC counts is not clear. The high degree of inter-rater agreeability further points towards the accuracy of our observations.

We also observed some discomforting points about the included studies. Seven of the 20 interventions studied^[19,20,25,28,32,34,36] were composite mixtures of a variety of Ayurvedic ingredients. By the nature of these individual studies, it is not possible to determine which component/s of these composite ingredients have prominent and contributory effect towards immune enhancement, and which components have secondary roles such as excipients, flavouring and colouring agents or other non-essential functions. On the other hand, the exact mechanisms by which the remaining thirteen individual Ayurvedic interventions impacted the immune system have been not adequately explored in the individual studies. Further contributing to the lack of clarity are the observations that there are some contradictory views wherein an apparent lack of effect in the results is claimed to have an effect (as in the case of cord blood IgG),^[32] or contradictory correlations given for opposite directions of alterations in blood cytokine levels.^[24,26] Next, as seen in **table 3**, as many as 6 studies have confusing, ambiguous, or incomplete results for various reasons. The sample size of the included studies is not sufficiently large enough. None of the 20 RCTs described in the 19 studies included in the present review was able to score a 'low risk of bias' rating in the RoB-2 tool, or had a mention of points indicative of well-conducted RCTs, such as protocol registration in a clinical trial registry, or having followed any reporting guidelines. All these factors generally tend to reduce confidence in the results presented in the studies, regardless of magnitude or statistical significance.

In the backdrop of these discouraging observations, it is apparent that, despite Ayurveda being an ancient science with an acclaimed and proven effect on immune system enhancement, and despite the availability of modern medical research methodologies and technological advancements, we have not been able to know exactly how such an enhancement in immune function is brought about through the consumption of a variety of Ayurvedic medicines. The proponents of Ayurveda mostly seem to rely on the historical anecdotes, rather than using the benefits of scientific advancements to improve the general confidence of the worldwide audience in this ancient science that has stood the test of time. While this lacuna on the part of

Ayurvedic researchers has been observed and commented upon for quite some time now,^[37] solid steps to enhance systematic evidence generation in Ayurveda seem to be lacking. In fact, ancient Ayurvedic texts have already incorporated many of the basic tenets of modern scientific discovery, such as *Anumana* (logical questioning), *Yukti* (knowledge), *Tarka* (argument), and *Vyapti* (cause and effect relationship).^[38] It should be realised that before the advent of modern medicine, Ayurveda was the 'modern medicine' of those times. Perhaps it is time that proponents of Ayurveda and modern medicine come together with open minds to apply modern scientific methods to generate credible and reproducible evidence, so that there is rational and evidence-based integration of Ayurveda into mainstream medicine. Also, it is apt that this is done by Indian scientists, rather than waiting for the Western scientists to do it on our behalf, as in the case of turmeric, neem, *Basmati* rice,^[39,40] *pranayama*-meditation,^[41] and many others.

While our initial search resulted in a pool of over 12,000 potentially relevant articles, we were able to identify only 19 RCTs that matched our eligibility criteria. During the process of literature screening, we observed that a large majority of research in this field is non-clinical, and largely done in-vitro, or on animal models. It appears that a meaningful translation to clinical research is lacking for many Ayurvedic preparations, whose efficacy and safety were established in non-clinical models, for reasons unknown. It appears that Ayurvedic researchers are restricting themselves from entering clinical research, and the reasons for this hinderance should be sought out and resolved, in order to boost clinical research in this field.

Our review should be interpreted in the backdrop of some limitations. We restricted to only papers published in English language, because of our familiarity with the language; we might have missed valid papers published in regional languages. We did not search other databases such as Embase or Scopus, since both these resources are paywalled, and our research was self-funded.

CONCLUSION

To conclude, various Ayurvedic preparations, both standalone and composite, appear to have an enhancing effect on the immune system, as evidenced indirectly through reduced illness variables, but the exact mechanism behind this enhancement is not fully established. There may be contributions from enhancement of NK cells and T helper cells, although the role of other immune system components is not clear. There were many inconsistencies and ambiguities with respect to the included studies. The numerous benefits of Ayurveda are being masked by a lack of proper research. The general need to improve the quality of research in Ayurveda is clearly visible, and the strong evidence thus generated, preferably by Indian researchers, will go a long way in fostering widespread acceptance of the immense knowledge of this ancient science by all stakeholders.

ARTICLE HIGHLIGHTS

Research background

Ayurveda is the Indian traditional system of medicine, and has a history since the 2nd century BC. Many Ayurvedic preparations have been anecdotally claimed to have immune-boosting properties, and have been used for immune enhancement and general well-being. Pre-clinical research suggests that the immune enhancement is mediated through multiple immune system modulation and psychoneuroimmunological mechanisms.

Research motivation

We were interested to know the exact mechanisms of immune enhancement by Ayurvedic preparations when they are administered to healthy or sick humans.

Research objectives

The objectives of the present systematic literature review (SLR) were to gather evidence towards the nature and mechanism of enhancement of human immune system by the

administration of Ayurvedic preparations, from published randomized clinical trials (RCTs).

Research methods

We prospectively registered the study protocol with PROSPERO. Based on predetermined eligibility criteria, search strategy was formulated and refined, and the same was used to search PubMed, DOAJ, Google Scholar, three dedicated Ayurveda research portals, two speciality Ayurveda journals, and reference lists for relevant records published until 02-06-2021. Baseline features and data pertaining to the nature and mechanism of immune system function were extracted from all eligible records. Methodological quality was assessed using the Cochrane RoB-2 tool.

Research results

Our search strategy yielded a total of 12,554 articles, and we found 19 studies reporting 20 RCTs (17 parallel group design, 3 crossover design) with 1,661 unique patients to be eligible for inclusion. Healthy population was included in 9 studies of which 1 study included pregnant women and 2 included paediatric population; remaining studies included patients with different health conditions. A total of 21 Ayurvedic interventions were studied, out of which 5 were composite mixtures. Through indirect evidence, four interventions were seen to be associated with immune enhancement, and two interventions were associated with a lack of such an enhancement. The role of T helper cell and NK cell enhancement was reported to contribute to the enhancement of immune systems by 3 and 4 interventions, respectively. Evidence pointing to enhancement of other immune system components, including cytotoxic T cells, B lymphocytes, immunoglobulins, cytokines, complement components, leucocyte counts, and other components, was not found. Risk of bias was 'high' in 9/20 RCTs, and 'some concerns' of bias were found in the remaining 11/20 RCTs, according to RoB-2.

Research conclusions

Various Ayurvedic preparations, both standalone and composite, appear to have an enhancing effect on the immune system, as evidenced indirectly through reduced illness variables, but the exact mechanism behind this enhancement is not fully established. There may be contributions from enhancement of NK cells and T helper cells, although the role of other immune system components is not clear.

Research perspectives

There is a need to improve the quality of research in Ayurveda. Ayurvedic scholars should team up with experts of modern clinical research and generate credible and reproducible evidence towards immune system enhancement. This will enable widespread acceptance of the immense knowledge of Ayurveda, leading to its increased usage, and ultimately, a healthier society.

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