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

B N Vallish, Dimple Dang, Amit Dang

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Abstract

BACKGROUND

Many Ayurvedic preparations are claimed to have immune-boosting properties, as suggested in various published randomized clinical trials (RCTs)

AIM

To compile evidence on the nature and mechanism of immune system enhancement by Ayurvedic preparations in healthy and sick individuals.

METHODS

After prospectively registering study protocol with PROSPERO, we searched PubMed, DOAJ, Google Scholar, three dedicated Ayurveda research portals, two specialty Ayurveda journals, and reference lists for relevant records published until February 6, 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 12554 articles screened, 19 studies reporting 20 RCTs (17 parallel group design, three crossover design) with 1661 unique patients were included; 11/19 studies had Indian first authors. Healthy population was included in nine studies, of which one study included pregnant women and two included pediatric population; remaining studies included patients with different health conditions, including one study with coronavirus disease 2019 patients. A total of 21 Ayurvedic interventions were studied, out of which five 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 five RCTs exploring five Ayurvedic interventions were incomplete, ambiguous, or confusing. Of the remaining 16 interventions, indirect evidence of immune enhancement was reported with four interventions, while lack of the same was reported with two interventions. Enhancement of T helper cells and natural killer cells was reported with three and four 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, particularly *via* enhancements in natural killer cells and T helper cells.

Key Words: Immune enhancement; Ayurveda; Immune system; Healthy volunteers; Composite preparations; NK cells

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Core Tip: Ayurvedic preparations have been anecdotally associated with immune boosting effect in both healthy and sick individuals. Through this systematic review, we explored the nature and mechanism behind this effect by scrutinizing 20 randomized controlled trials reported in 19 articles. While we could find indirect evidence for immune enhancement (by means of reduced illness duration and severity) with some Ayurvedic preparations, the evidence was insufficient to conclude about the exact mechanisms contributing to this phenomenon, although available evidence suggests that enhancements in natural killer cells and T helper cell number and function might contribute.

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INTRODUCTION

As of September 2021, the ongoing coronavirus disease 2019 (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 severe acute respiratory syndrome coronavirus 2 (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 of the immune system are reported to exacerbate paradoxically 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 humors (*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

Study protocol

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

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 pediatric 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[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.

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 June 2, 2021. Recognizing that many research articles on Ayurveda are published in journals not indexed in PubMed, and following the recommendations by Aggithaya *et al*[10] (2015), we performed an additional literature search in Directory of Open Access Journals[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 specialty 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 in [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, and 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, natural killer (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 (VBN and 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).

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 (Armonk, NY, United States). 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 (VBN). All datasets used to derive conclusions in this study are available with the corresponding author on reasonable request.

RESULTS

Study selection, baseline characteristics

From an initial pool of 12554 potentially eligible records, 19 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 six different countries, with India being the most frequent country of affiliation (11 studies), followed by Japan and United States (two studies each); there was one study each with first authors from Germany, Iran, Romania, and Turkey. The participant profile ranged from healthy participants (six studies, healthy adults; two studies, healthy pediatric population; one study, healthy pregnant women), patients with cancer (three studies, gastrointestinal cancer; one study, head and neck cancer), patients with diabetes and human immunodeficiency virus (HIV) (two studies each), and patients with COVID-19 and allergic rhinitis (one 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 1661 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 five studies, and *Rasayana* in three 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 six 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

Table 1 Baseline study characteristics, and summary of risk of bias assessment

No	Ref.	Country of 1 st author	Type of randomized controlled trial	Patient profile	Intervention ¹			Comparator ¹			Overall risk of bias as per RoB-2 tool ²
					Sample size	Mean age	Male (N, %)	Sample size	Mean age	Male (N, %)	
1	Enesel <i>et al</i> [18], 2005	Romania	Parallel group	Patients undergoing surgery for GIT cancer	40	62 (range 37-87)	23 (57.5)	30	NA	NA	-
2	Ishikawa <i>et al</i> [21], 2006	Japan	Parallel group	Patients with inoperable colon/liver/pancreatic cancer	25	63.6 ± 8.3	21 (84)	25	65.8 ± 6.3	18 (72%)	?
3	Brush <i>et al</i> [22], 2006	United States	Parallel group	Healthy adult volunteers	3	NA	NA	2	NA	NA	-
4	Schink <i>et al</i> [27], 2007	Germany	Parallel group	Primary/locally relapsed colorectal carcinoma patients undergoing open (complete/partial) tumour resection	11	72 ± 8.2	7 (63.6)	11	69 ± 10.4	5 (45.5)	?
5	Purandare <i>et al</i> [33], 2007	India	Parallel group	Patients with diabetic foot ulcer	23	56.26	17 (73.9)	22	56.32	19 (86.4)	?
6	Uebaba <i>et al</i> [29], 2008	Japan	Crossover	Healthy adult female volunteers	16	39 ± 9	0 (0)	16	39 ± 9	0 (0)	?
7	Bhat <i>et al</i> [28], 2010	India	Parallel group (I)	Healthy adult volunteers	13	NA	NA	13	NA	NA	?
			Crossover (II)	Healthy adult volunteers	110	NA	NA	110	NA	NA	-
8	İşik <i>et al</i> [23], 2010	Turkey	Parallel group	Allergic rhinitis patients with house dust mite sensitivity	12	NA	NA	12	NA	NA	-
9	Kianbakht <i>et al</i> [30], 2011	Iran	Parallel group	Healthy adult male volunteers	45	22.5 ± 0.6	45 (100)	44	21.1 ± 0.5	44 (100)	?
10	Mondal <i>et al</i> [24], 2011	India	Crossover	Healthy adult volunteers	22	NA	NA	22	NA	NA	?
11	Nantz <i>et al</i> [26], 2012	United States	Parallel group	Healthy adult volunteers	56	25.4 ± 5.7	23 (41.1)	56	26.9 ± 7.1	26 (46.4)	?
12	Suprabha <i>et al</i> [32], 2017	India	Parallel group	Uncomplicated pregnant women in 20-24 wk of pregnancy	15	NA	NA	15	NA	NA	?
13	Gupta <i>et al</i> [34], 2017	India	Parallel group	Healthy children aged 5-12 yr	313	7.3 ± 1.8	161 (51.4)	314	7.4 ± 1.8	164 (52.2)	?
14	Rais <i>et al</i> [36], 2021	India	Parallel group	25-60 yr, asymptomatic/uncomplicated COVID-19 RTPCR +ve, mild symptoms	80	NA	57 (71.3)	40	NA	30 (75)	-
15	Bhaskaran <i>et al</i> [31], 2019	India	Parallel group	Healthy full-term infants (< 12 mo age), > 2.5 kg birth weight, with normal growth and development	47	NA	NA	34	NA	NA	-
16	Kumar <i>et al</i> [35], 2014	India	Parallel group	Adult patients with T2DM of any stage	56	NA	NA	28	NA	NA	-
17	Ravindran <i>et al</i> [25], 2014	India	Parallel group	Patients with head & neck cancer in complete remission following primary treatment	37	NA	NA	38	NA	NA	?
18	Somarathna <i>et al</i> [20], 2010	India	Parallel group	HIV +ve patients without AIDS surveillance signs as per WHO, and no concurrent illness	21	NA	NA	6	NA	NA	-
19	Gupta <i>et al</i> [19], 2010	India	Parallel group	New HIV +ve patients with CD4 count not < 150/microliter and no complications or comorbidities	12	NA	NA	8	NA	NA	-

¹Not all studies had reported demographics of participants including age and sex.

²Separate versions of RoB-2 were used for RCTs with parallel group design and crossover design. Interpretation: ?: Some concerns of bias; -: High risk of bias. Detailed risk of bias assessment results: [Supplementary Table 4](#). HIV: Human immunodeficiency virus; NA: Not available; GIT: Gastrointestinal tract; COVID-19: Coronavirus disease 2019; RTPCR: Reverse transcription polymerase chain reaction.

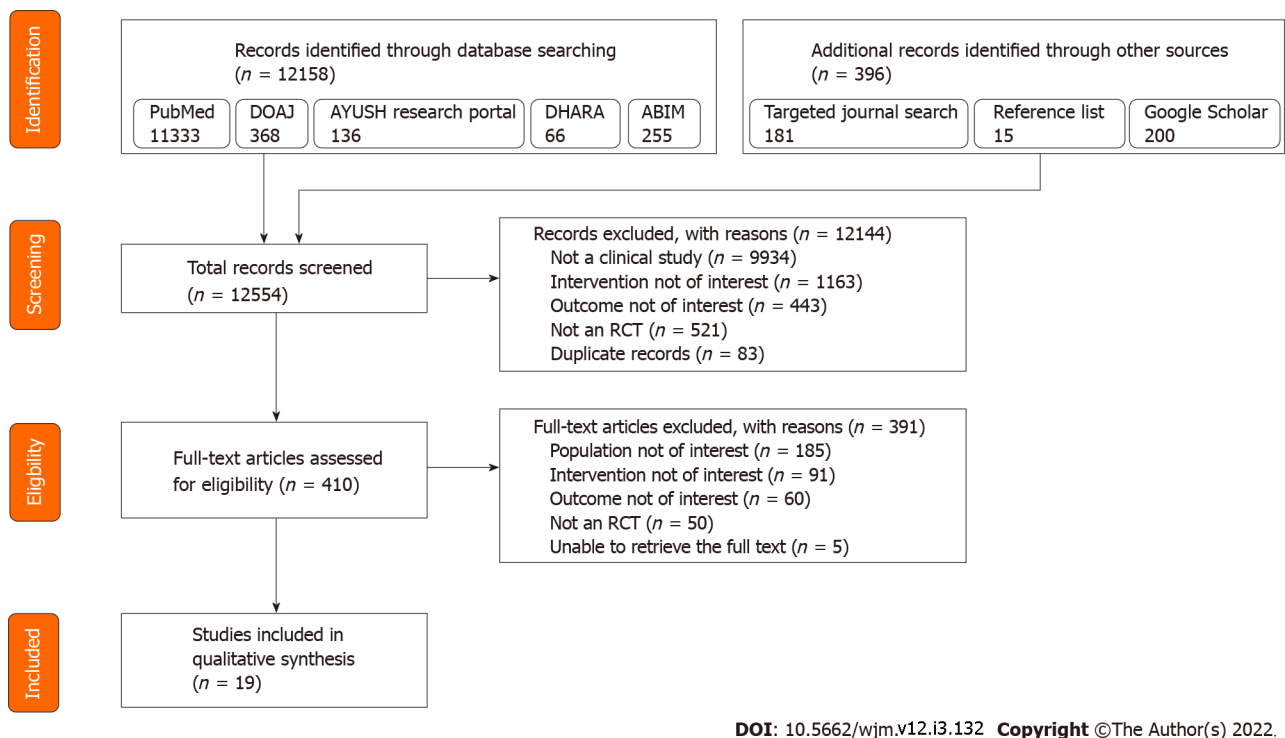


Figure 1 Study selection process. DOAJ: Directory of Open Access Journals; AYUSH research portal: Ayurveda, Yoga, Unani, Siddha, and Homoeopathy research portal; DHARA: Digital Helpline for Ayurveda Research Articles; ABIM: Annotated Bibliography of Indian Medicine.

five of the studies is provided in [Supplementary Table 3](#).

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), three 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.

Immune enhancement: Direct evidence

Impact on T lymphocyte subsets (excluding NK cells): CD4 lymphocyte counts were found to be significantly enhanced after perioperative mistletoe administration for 14 d among patients undergoing surgery for gastrointestinal (GI) cancer[18]. Among patients with HIV, CD4 lymphocyte counts were found to be enhanced after a 90-d 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 5 patients[22]. Significant enhancement of CD4 lymphocyte count was also not observed with *Nigella sativa* seed supplementation, and the data were 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 d[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: although *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 5 patients[22]. Significant CD8 lymphocyte count enhancement was observed with *Nigella sativa* seed

Table 2 Details of intervention and comparators

No	Ref.	Description	Intervention: Ayurvedic name ¹	Intervention: Scientific name	Intervention: Route; dose, frequency, duration ²	Comparator: Description; route, dose, frequency, duration ²
1	Enesel <i>et al</i> [18], 2005	Isorel® (Mistletoe, firtree)	Bandaaka, Suvarna-bandaaka <i>etc.</i>	<i>Viscum album</i>	Subcutaneous; 2 wk pre-operatively, 2 wk post-operatively; dose details not clear	Standard care
2	Ishikawa <i>et al</i> [21], 2006	Aged garlic extract	Lashuna, Rasona <i>etc.</i>	<i>Allium sativum</i>	Oral; 125 mg, 4 capsules daily, 12 wk	Matching placebo
3	Brush <i>et al</i> [22], 2006	<i>Glycyrrhiza glabra</i> tincture	Yashtimadhu, Madhuyashtyaahvaa <i>etc.</i>	<i>Glycyrrhiza glabra</i>	Oral; 0.44 g/7.5 mL, twice daily, 7 d	Matching placebo tincture
4	Schink <i>et al</i> [27], 2007	Standard care + Iscador® (Standardized mistletoe extract)	Bandaaka, Suvarna-bandaaka <i>etc.</i>	<i>Viscum album</i>	IV infusion; 5 mg, single dose	Standard care
5	Purandare <i>et al</i> [33], 2007	Standard care + <i>Tinospora cordifolia</i>	Guduuchi, Guduuchikaa <i>etc.</i>	<i>Tinospora cordifolia</i>	Details not available	Standard care + matching Placebo
6	Uebaba <i>et al</i> [29], 2008	Shirodhara oil-dripping treatment using sesame oil	Shirodhara; Tila, Snehpala	<i>Sesamum indicum</i>	Oil dripping on forehead; single sitting	Control supine position; single sitting
7	Bhat <i>et al</i> [28], 2010	Fortified tea with <i>Withania somnifera</i> , <i>Glycyrrhiza glabra</i> , <i>Zingiber officinale</i> , <i>Ocimum sanctum</i> & <i>Elettaria cardamomum</i>	Ashwagandha; Yashtimadhu; Aardraka, Shunthi; Tulasi; Elaa	<i>Withania somnifera</i> , <i>Glycyrrhiza glabra</i> , <i>Zingiber officinale</i> , <i>Ocimum sanctum</i> , <i>Elettaria cardamomum</i>	Oral; 2.06 g, thrice daily, 2 mo	Regular tea; Oral; 2 g, thrice daily, 2 mo
8	Işik <i>et al</i> [23], 2010	Specific immunotherapy + <i>Nigella sativa</i>	Kaalaajaaji, Kalikaa <i>etc.</i>	<i>Nigella sativa</i>	Oral; 2 g daily, 1 mo	Specific immunotherapy alone
9	Kianbakht <i>et al</i> [30], 2011	Saffron tablet	Kumkuma, Rudhira, Kaashmiraka <i>etc.</i>	<i>Crocus sativus</i>	Oral; 100 mg daily, 6 wk	Matching placebo
10	Mondal <i>et al</i> [24], 2011	Tulsi capsules	Tulasi, Surasa, Suravalli <i>etc.</i>	<i>Ocimum sanctum</i>	Oral; 300 mg daily, 4 wk	Matching placebo
11	Nantz <i>et al</i> [26], 2012	Aged garlic extract	Lashuna, Rasona <i>etc.</i>	<i>Allium sativum</i>	Oral; 640 mg, 4 capsules daily, 90 d	Matching placebo
12	Suprabha <i>et al</i> [32], 2017	Rasayana Avaleha with milk	Composite ⁴	Composite ⁴	Oral; 12 g, twice daily, 2 mo	Calcium carbonate (500 mg) with ferrous sulfate (200 mg); Oral; daily, 2 mo
13	Gupta <i>et al</i> [34], 2017	Cyavanaprasa (Dabur) with milk	Composite ⁴	Composite ⁴	Oral; 6 g, twice daily, 6 mo	Milk; Oral, 100-200 mL, twice daily, 6 mo
14	Rais <i>et al</i> [36], 2021	Intervention 1: Vyaghryadi Kashaya (50 mL) + Pippali (250 mg) + Samshamani vati (500 mg) Intervention 2: Shunthi churna (2 g) + Rasona kalka (1 g)	Vyaghryadi Kashaya: Kantakari, Shunthi, Guduchi; Pippali; Samshamani vati: Guduchi Shunthi; Rasona, Lasuna	<i>Solanum xanthocarpum</i> , <i>Zingiber officinale</i> , <i>Tinospora cordifolia</i> , <i>Piper longum</i> <i>Zingiber officinale</i> , <i>Allium sativum</i>	Oral; twice daily, 10 d Oral; Shunthi churna: Twice daily, Rasona: once daily; 10 d	Vitamin C (500 mg) twice daily, Paracetamol (500 mg) as needed
15	Bhaskaran <i>et al</i> [31], 2019	Swarna Bhasma (Calcined powder of gold), honey, ghrita	Swarna prashana, madhu, ghrita	NA	Oral; Swarna bhasma: 0.2-2.4 mg ³ ; once daily, 4 wk	Oral Honey + ghrita; dose details not available
16	Kumar <i>et al</i> [35], 2014	Intervention 1: Mamajjaka capsules Intervention 2: Shilajatu capsules	Maamajjaka, Naagihvaa <i>etc.</i> Shilajatu	<i>Enicostemma littorale</i> <i>Asphaltum punjabinum</i>	Oral; 500 mg, twice daily, 3 mo Oral; 500 mg, twice daily, 3 mo	Control; no details available
17	Ravindran <i>et al</i> [25], 2014	Varunadi Ghrita + Standard care	Composite ⁴	Composite ⁴	Oral; 5 g, twice daily, 1 y	Standard care
18	Somarathna <i>et al</i> [20], 2010	Ranahamsa Rasayana	Composite ⁴	Composite ⁴	Oral; 5 g, twice daily, 90 d	Standard care
19	Gupta <i>et al</i> [19], 2010	Shilajatu Rasayana	Composite ⁴	Composite ⁴	Oral; 95 g over first 15 d, later 6 g per day for	Standard care

¹Out of various Ayurvedic names, only two are mentioned.²d: Day; wk: Week; m: Month; yr: Year.³Dosage varied based on age of infant.⁴Detailed composition: [Supplementary Table 3](#). IV: Intravenous.

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 d 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].

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 d among patients undergoing surgery for GI cancer [18] and also by consumption of aged garlic extract capsules for 12 wk among patients with inoperable GI 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 d of surgery with a single-dose mistletoe extract intravenous 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 percentage, 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-min *Shirodhara* treatment with sesame oil was associated with a significant enhancement in NK cell activity compared to the control supine position for 30 min[29], and among healthy volunteers, the consumption of aged garlic extract for 45 d 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 substantiate fully these claims.

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 three studies had methodological or reporting issues. Next, perioperative consumption of mistletoe extract was associated with a significant increase in serum levels of immunoglobulin (Ig)A 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 fetal immunity level, despite absence of strong evidence pointing towards the same.

Impact on complement components and cytokines: While serum levels of complement C3 and C4 proteins were found to increase significantly after 14 d 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 d was associated with non-significantly reduced serum levels of tumor necrosis factor α and interferon 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 increase significantly the levels of interferon gamma and interleukin-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.

Impact on white blood cell counts and granulocytes: Even though total white blood cell (WBC) counts were enhanced by perioperative mistletoe administration after 14 d, 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], or 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 enhance significantly 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 d among patients with HIV[20]. Phagocytic function by neutrophils was found to be significantly enhanced after a 1-mo 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 decrease significantly after a 4-wk 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].

Impact on other immune system variables: Perioperative subcutaneous mistletoe administration over 14 d was found to increase significantly the counts of CD2 lymphocytes (comprising of T lymphocytes and NK cells) after 14 d 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 human leukocyte antigen - DR isotype expression, which was also seen among patients who received standard care[27].

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 d 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 signifies 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 reverse transcription polymerase chain reaction results was zero, after 10 d of treatment with either *Vyagradhi Kashaya*, ginger-garlic, or Vitamin C[36].

The complete details of the main findings of all the included studies are summarized in Table 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*[36] reported that 2/40 patients

Table 3 Results from the included studies: Impact of Ayurvedic medication on components of the immune system

No	Ref.	Intervention	T Helper cells	T cytotoxic cells	T cells: Other results	NK cell count/activity	B lymphocyte count	Immuno-globulins	Comple-ment	Cyto-kines	WBC count/activity	Other variables	Indirect evidence
1	Enesel <i>et al</i> , 2005	Isorel® (Mistletoe, firtree)	+	-	+	+	-	+	+	NA	+	+	NA
2	Ishikawa <i>et al</i> , 2006	Aged garlic extract	-	-	NA	+	NA	NA	NA	NA	-	NA	NA
3	Brush <i>et al</i> , 2006 ¹	<i>Glycyrrhizia glabra</i> tincture	+/-	+/-	NA	+/-	+/-	NA	NA	NA	NA	NA	NA
4	Schink <i>et al</i> , 2007	Iscador® (Standardized mistletoe extract)	NA	NA	NA	+	NA	NA	NA	NA	NA	-	NA
5	Purandare <i>et al</i> , 2007	Tinospora cordifolia	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA
6	Uebaba <i>et al</i> , 2008 ²	Shirodhara oil-dripping (sesame oil)	NA	NA	NA	+/-	NA	NA	NA	NA	NA	NA	NA
7	Bhat <i>et al</i> , 2010 ³	Fortified tea with multiple Ayurvedic ingredients	NA	NA	NA	+/-	NA	NA	NA	NA	NA	NA	NA
8	Işik <i>et al</i> , 2010	Specific immunotherapy + <i>Nigella sativa</i>	+/-	+/-	+/-	+/-	+/-	NA	NA	NA	+	NA	NA
9	Kianbakht <i>et al</i> , 2011	Saffron tablet	NA	NA	NA	NA	NA	-	-	NA	-	NA	NA
10	Mondal <i>et al</i> , 2011 ⁴	Tulsi capsules	+/-	+/-	NA	+/-	+/-	NA	NA	+/-	NA	NA	NA
11	Nantz <i>et al</i> , 2012 ⁵	Aged garlic extract	NA	NA	+/-	+/-	NA	NA	NA	+/-	NA	NA	+
12	Suprabha <i>et al</i> , 2017	Rasayana Avaleha with milk	NA	NA	NA	NA	NA	-	NA	NA	NA	NA	NA
13	Gupta <i>et al</i> , 2017	Cyavanaprasa (Dabur) with milk	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+
14	Rais <i>et al</i> , 2021	Intervention 1: Vyaghryadi Kashaya + Pippali + Samshamani vati	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
		Intervention 2: Shunthi churna + Rasana kalka	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
15	Bhaskaran <i>et al</i> , 2019 ⁶	Swarna Bhasma, honey, ghrita	NA	NA	NA	NA	NA	+/-	NA	NA	+/-	NA	NA
16	Kumar <i>et al</i> , 2014	Intervention 1: Mamajjaka capsules	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+
		Intervention 2: Shilajatu capsules	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+

17	Ravindran <i>et al</i> , 2014	Varunadi Ghrita + Standard care	NA	NA	+	+	+	NA	NA	NA	NA	NA	NA
18	Somarathna <i>et al</i> , 2010	Ranahamsa Rasayana	+	NA	NA	NA	NA	NA	NA	NA	-	NA	NA
19	Gupta <i>et al</i> , 2010	Shilajatu Rasayana	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SUMMARY		+	3	0	2	4	1	1	1	0	3	1	4
		+/-	3	3	2	6	3	1	0	2	1	0	0
		-	1	2	0	0	1	2	1	0	3	1	2

+: Evidence suggests significant immune enhancement; +/-: Ambiguous, confusing, or incomplete results; -: Evidence does not suggest significant immune enhancement.

¹Sample size is 5 participants; significance values of any results not reported in the paper.

²paper reports only significance (*P* value) without reporting the value of NK cell activity.

³study I (parallel group study design) reports only significance (*P* value) without reporting the value of NK cell activity, and study II (crossover design) has less than optimal washout period, and there is a difference in numbers between text and table.

⁴paper reports only significance (*P* value) without reporting the value of T lymphocytes, B lymphocytes, NK cells, and cytokines.

⁵paper reports only significance (*P* value) without reporting the value of T lymphocytes and NK cells.

⁶paper reports only significance (*P* value) and mean difference without reporting the value of immunoglobulins and total or differential white blood cell counts.

NA: Not available; NK: Natural killer; WBC: White blood cell.

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. 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].

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 three 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 in Supplementary Table 4.

IRR

The IRR for study selection between the two reviewers was substantial, with Cohen's kappa value being 0.743 [95% confidence interval (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,

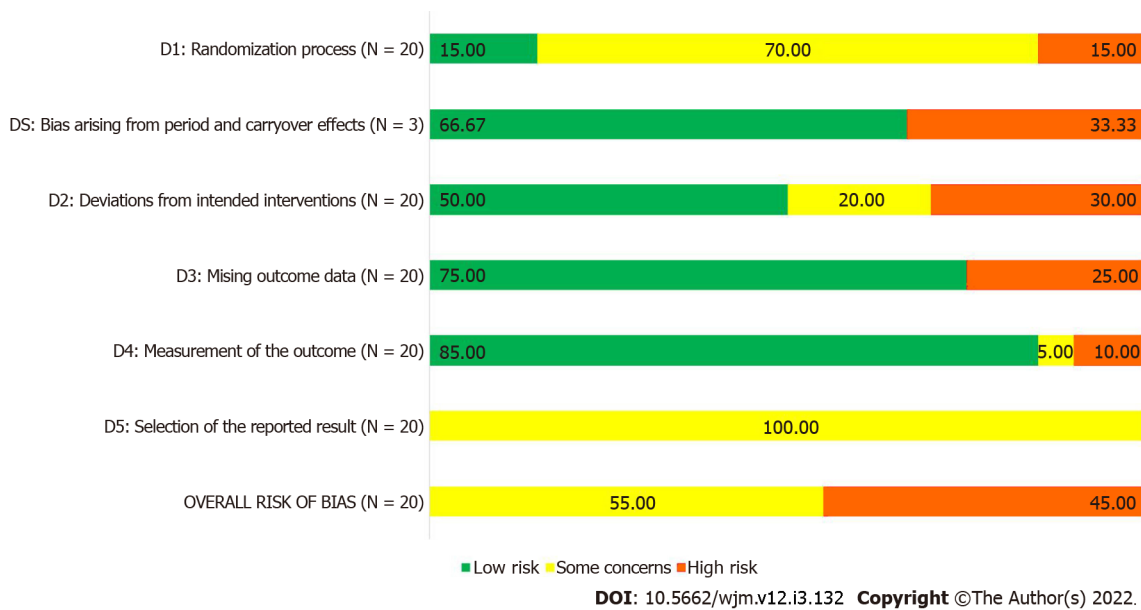


Figure 2 Risk of bias summary of the included studies, as per RoB-2 tool.

with four 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, flavoring and coloring agents or other non-essential functions. On the other hand, the exact mechanisms by which the remaining 13 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 six 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, had a mention of points indicative of well-conducted RCTs, such as protocol registration in a clinical trial registry, or had 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 realized 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 12000 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 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 specialty Ayurveda journals, and reference lists for relevant records published until February 6, 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 12554 articles, and we found 19 studies reporting 20 RCTs (17 parallel group design, three crossover design) with 1661 unique patients to be eligible for inclusion. Healthy population was included in nine studies, of which one study included pregnant women and two included pediatric population; remaining studies included patients with different health conditions. A total of 21 Ayurvedic interventions were studied, out of which five 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 natural killer cell enhancement was reported to contribute to the enhancement of immune systems by three and four 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 natural killer 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.

FOOTNOTES

Author contributions: Vallish BN contributed to acquisition of data, analysis and interpretation of data, drafting the article, making critical revisions related to important intellectual content of the manuscript, and final approval; Dang D contributed to acquisition of data, analysis and interpretation of data, making critical revisions related to important intellectual content of the manuscript, and final approval; Dang A contributed to conception and design of the study, acquisition of data, making critical revisions related to important intellectual content of the manuscript, and final approval.

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