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Pre-clinical studies of Siddha formulations advocated for anaemia: A systematic review and meta-analysis

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ABSTRACT

Anaemia is a global burden that affects millions of people's health and quality of life. It is one of the most severe and important nutritional deficiencies prevailing in the world. Siddha, a known system of Indian Medicine owns numerous medicinal preparations for the treatment of anaemia with literature evidences in many classical texts. To date, many clinical and pre-clinical trials have been carried out in Siddha formulations which were indicated for anaemia. This manuscript deals with the systematic review and meta-analysis of pre-clinical studies of various Siddha formulations used for the treatment of anaemia. The aim of the study was to compile and summarize the findings of all relevant pre-clinical studies of Siddha formulations used for treating anaemia. Data of pre-clinical trials of various haematinic Siddha formulations were retrieved from electronic databases like PubMed, Cochrane, Science Direct, Medline, Scopus, and Google scholar. Preferred reporting items for systematic review and meta-analysis guidelines were adopted for systematic review and RevMan 5.2 software was used for the meta-analysis. Systematic review demonstrated the difference in haemoglobin (Hb), red blood corpuscles (RBC) count and heavy metals levels in these Siddha herbal and herbo-mineral/herbo-metallic formulations. Meta-analysis has revealed significant increase in Hb and also in total RBC count after treatment with these Siddha Haematinics. The meta-analysis of toxicity studies also reveals that no toxic effects were observed up to the level of therapeutic dose. The outcome of this systematic review and meta-analysis demonstrated that great number of Siddha formulations has been available since ages for the treatment of anaemia and the same has been proved by various evidence based safety and efficacy studies. However, a well-designed randomized clinical trials of these Siddha interventions are need of the hour for the recognition of this traditional system of medicine in the treatment of anaemia globally.

INTRODUCTION

Anaemia, constitutes a major public health concern in developing countries. According to WHO global statistics, 40% of pregnant women, and 42% of children under 5 are *anaemic*. It alters immune mechanisms and also associated with increased morbidity rates. The economic and social growth of a nation is impacted by anaemia, as it is attributed to delayed cognitive and motor development in children and reduced work capacity in adults (WHO, 2022). Additionally, anaemia during pregnancy is also associated with increased risk of haemorrhage, sepsis, maternal mortality, perinatal mortality, and has poor reproductive outcomes such as preterm birth, low-birth-weight babies and diminished iron reserves for the foetus, etc. It is estimated that approximately all women are iron deficient to some degree and more than half of the pregnant women suffer from anaemia in developing countries. Millions of individuals may suffer from poor health and quality of life if the prevalence of anaemia is not reduced as this would ultimately hinder the development of a nation. The recently released National Family Health Survey (NFHS-5) census reveals that the prevalence of anaemia has increased to 62.6% among children, women of all age groups including pregnant women and men in India [Release of NFHS-5 (2019-21)].

Although the traditional treatment for iron deficiency anaemia involves taking iron supplements like ferrous sulphate or elemental iron, adherence to the medicine is challenging due

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to variety of adverse side effects like metallic taste, epigastric discomfort, nausea, diarrhea, or constipation, etc. The adverse effects of iron supplements can be reduced by taking medications with food. However, doing so might decrease the absorption of iron. Therefore, there is a need to search for innovative drugs that have better therapeutic value and lesser side effects (Nguyen and Tadi, 2022).

Siddha, a well-known system of Indian medicine owns numerous medicinal preparations for the treatment of anaemia since ages. Siddha medicines are of 64 types viz. 32 internal medicines and 32 external medicines (Rajendra Kumar *et al*., 2018). All these medicinal formulations were prepared by using plant (herbs), minerals, metals, and animal products. Many types of Siddha formulations like *Chooranam, Vadagam, Manappagu, Paanidham, Nei, Mathirai, Elagam, Karpam, Chendhuram, etc* of Polyherbal, herbo-mineral/ herbo-metallic origin are available for the treatment of anaemia (Thirunarayanan, 2012). So far, many clinical trials including few randomized clinical trials (RCTs), pre-clinical studies, and other studies have been carried out in these Siddha formulations for the correction of anaemia.

Pre-clinical trials provide the first insight on drug's suitability for testing on humans by ensuring safety and efficacy in *in-vitro*, *in-vivo* or *in-silico* studies. Therefore, pre-clinical studies of Siddha formulations form the objective of current study in order to compile the data of various studies done so far.

Since, all these Siddha formulations have lesser or minimal side effects, they are successfully used by Siddha Physicians for the treatment of anaemia widely. More so, a medicinal kit including *Madhulai Manappagu* and *Annabedhi Chendhuram* was provided by Primary Healthcare Centers to each pregnant women under Tamil Nadu State Government's Health Program "*Amma Magapperu Sanjeevi Thittam*" to prevent anaemia during pregnancy since 2016. Therefore, it is the need of the hour to take this traditional Indian system of medicine for its global recognition in anaemia treatment. Hence this study was aimed to conduct from all the available data from 1982 to till date. The objectives of this study includes compilation and summation of all the findings of relevant pre-clinical studies of Siddha formulations in the treatment of anaemia. It also emphasizes the strength of evidence in treating anaemia with Siddha formulations. The outcome of the study may throw light on the efficacy of various Siddha formulations and also will explore the scope of future research prospects in this traditional system of medicine to combat anaemia.

METHODOLOGY

This systematic review was carried out in accordance with the principles of Cochrane Collaboration framework and also by following the guidelines of Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA).

Literature search strategy

Using text word search strategy, the literature search was carried out in electronic databases. Online platforms like PubMed, Cochrane, ScienceDirect, Medline, Scopus, and Google scholar were used in search of articles. The search terms include *pandu, paandu, pantu, veluppu, veluppu noi, and sogai*. The search was also elaborated by using the search terms *annabethi or annabedhi or annabedi, karisalai or karislankanni or karisaalai, ayam, kantham/kaantham/kandham/gandham, maathulai/madhulai* to extract a greater number of studies (The search words were selected based on some common Siddha formulations used for anaemia and its correction). To maximize the sensitivity of the search and to avail most of the relevant studies, general keywords like Iron deficiency anaemia *and* Siddha, Anaemia and Siddha, Anemia and Siddha, Sittha medicine and anaemia were also used.. Furthermore, bibliographies from the extracted studies were also reviewed and researched to identify additional studies.

No limitations were set during the literature search. The literature search was carried out with a time span of about 40 years i.e., from 1982–2022 and they were collected from July to September 2022.

Study selection criteria

The criteria followed for individual studies to be included in this systematic review and meta-analysis are as follows:

The study should be published in English language; published in indexed or peer-reviewed journals; adhered to the Siddha system of Indian medicine; with original data; pre-clinical studies with Siddha interventions; Studies with control and trial groups; Studies with quantitative estimation of Hemoglobin (Hb) between trial and control groups were considered eligible for meta-analysis. The trial results which discuss red blood corpuscles (RBC) studies and other blood investigations along with Hb were also included. If data was not summarized and discussed in the literature, raw data were extracted and statistical analysis was done by the authors to avail the maximum number of studies for meta-analysis.

The studies which did not fulfill the inclusion criteria were excluded. After title and abstract screening, some articles were excluded. Studies with non-available full text articles were also excluded. Furthermore, some articles with poor description of methodology, and statistical methods were excluded after full text article screening.

Data selection and extraction

The selection of studies for this systematic review and data extraction from eligible studies were done manually. Data were extracted from the studies and fetched in excel spreadsheets according to the requirements in different sections. General data including the name of authors, title, literature reference in classical Siddha texts, year of publication and study place were extracted primarily. Other than that, data like number of animals used, type of experimental model used, method of induction of anaemia, route of administration, details of intervention and outcome measures were also collected. For the meta-analysis, the quantitative data of Hb, RBC and other available parameters were entered separately.

Statistical analysis

Collected data were analyzed according to the requirements of the meta-analysis. The Cochrane collaboration tool, Rev-Man 5.2 software was used to carry out the study's statistical analysis. Pre-clinical Pharmacological studies were only used in meta-analysis. Standard mean deviation, 95% of Confidence interval and *p*-value were considered as summary statistics of this study. Heterogeneity was calculated using Cochrane's statistics (I^2). If the data is homogenous ($I^2 \leq 50\%$), meta-analysis was done using fixed effect model. In case of heterogeneous data ($I^2 \geq 50\%$), random effect model of meta-analysis was used. Publication bias

was addressed using funnel plot analysis. The *z*-score with a *p*-value less than 0.05 was considered statistically significant.

RESULTS

Description of included studies

About 79 articles were retrieved from a total of 975 after duplicates removal and title and abstract screening. 3 articles were excluded as their full texts were not available. 35 studies were excluded after full text screening. Finally, this systematic review included a total of 39 papers. Two articles (Padmagreesan, 2008; Shenbagavalli, 2009) were considered twice as they discussed two different formulations for anaemia in pre-clinical aspect. Hence, a total of 41 Siddha formulations with pre-clinical studies were included for systematic review. Among these, pre-clinical pharmacological data were extracted from 33 articles and pre-clinical toxicological data were extracted from 30 articles.

Meta-analysis was carried out in pre-clinical pharmacological data. Out of 33 articles, only 28 studies were found to be eligible for meta-analysis as 5 articles were excluded due to poor description of statistical data. Selection of articles for this review was shown as flowchart in Figure 1 which was designed based on PRISMA 2020.

Systematic review

Characteristics of included studies

Pre-clinical pharmacological studies

A total of 153 albino rats or mice of both sex and standard weight were used as experimental animals in all the studies. The chemical compound Phenyl hydrazine (*n* = 15) was used to induce anaemia artificially in experimental animals, other (*n* = 18) studies did not mention about the induction procedure. In all the studies, the oral route was adopted for the administration of medicines. The duration of treatment varies from 28 days to 42 days in pre-clinical pharmacological studies. Detailed characterization of included pre-clinical studies of both polyherbal and herbo-mineral/ herbo-metallic formulations were presented in Table 1.

Pre-clinical toxicological studies

Wistar albino rats or mice of both sex and standard weight according to Organization for Economic Cooperation and Development guidelines were used as experimental animals in all the studies. In all the acute toxicity studies, single oral gavage of trial drug maximum at the dose of 10× were given and observed for 14 days. In most of sub-acute/ repeated oral toxicity studies, oral administration of trial drugs at the doses of 1×, 3×, 5× and 10× were given for the period of 28 days/90 days and observed. Detailed characterization of included pre-clinical toxicological studies of polyherbal and herbo-mineral/ herbo-metallic drugs were presented in Table 2.

Study interventions and systematic review

Pre-clinical pharmacological studies

In this review article, 33 Siddha formulations which were used in the treatment of anaemia were included. Out of these 33 formulations, 19 were polyherbal formulations [*Chooranam* (*n* = 12); *Manappagu* (*n* = 2); *Nei* (*n* = 1); *Mathirai* (*n* = 1); *Elagam* (*n* = 1); *Karpam* (*n* = 1); *Usidham* (*n* = 1)] and 14 were herbo-mineral/herbo-metal formulations [*Vadagam* (*n* = 1); *Paanidham* (*n* = 1); *Mathirai* (*n* = 2); *Elagam* (*n* = 1); *Karpam* (*n* = 1); *Chenthuram* (*n* = 8)] as depicted in Figure 2. Some formulations were subjected to two or more pre-clinical trials as interventions by different authors to analyze its therapeutic efficacy, for example, in polyherbal formulations, *Karisalankanni Chooranam, Madhulai Manappagu* were analyzed in 2 different studies and in herbo-mineral formulations, *Annabedhi Chenduram, Thiripalai Mathirai* were used by 2 authors as interventional drug in their studies. Statistical data of Hb and RBC between trial and control groups as discussed in Table 1.

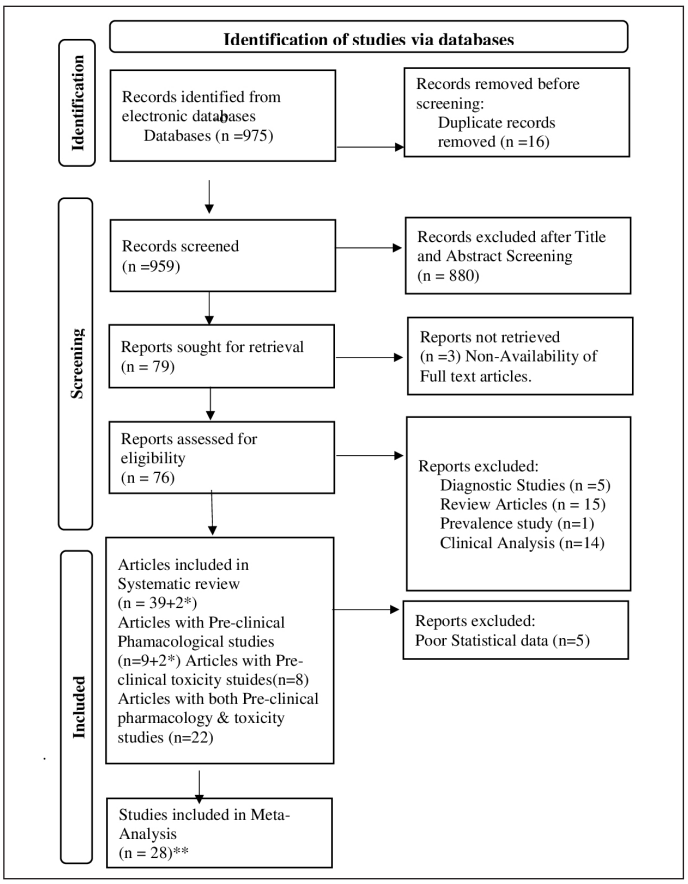

Figure 1. Prisma flow diagram of included studies, *-No. of studies considered twice. **- Only pre-clinical pharmacological studies considered.

Pre-clinical toxicological studies

About 30 studies were included and analyzed for toxicity evaluation of Siddha formulations. Acute Toxicity studies were carried out in all 30 Siddha formulations and Sub-Acute or Repeated Oral toxicity studies were carried out in 20 formulations only. Of all the Siddha formulations used in toxicity studies, 16 were polyherbal formulations [*Chooranam* (*n* = 11); *Manappagu* (*n* = 1); *Nei* (*n* = 1); *Mathirai* (*n* = 1); *Elagam* (*n* = 1); *Karpam* (*n* = 1)]; and 14 were herbo-mineral/herbo-metal formulations [*Paanidham* (n = 1); *Vadagam* (n = 1); *Mathirai* (n = 1); *Elagam* (n = 1); *Chenthuram* (n = 10)] as depicted in Figure 3.

Heavy metal contents in trial drugs

Out of 33 studies which were included in systematic review, 9 studies have evidenced the presence of minerals and

Table 1. Detailed description and characterization of included pre-clinical pharmacological studies.

S.No	Reference	Trial drug	Siddha literature	No. of groups Intervention period		Groups- intervention type and dosing	After treatment	
							Hb (g/dl) (Mean ± SD)	RBC (×l0^6/ul) (Mean ± SD)
Preclinical pharmacological (Efficacy) studies-poly herbal formulations (*n* = 19)								
1	Shenbagavalli (2009)	*Bringaraja Chooranam*	NM*	3	NM*	I- Control	5.2 ± 0.21	-
						II- Honey (Vehicle)	6.2 ± 0.29	-
						III- Trial drug at the dose 100 mg	8.8 ± 0.31	-
2	Kalaiselvi (2017)	*Chitramutti Nei*	*Chikicha Rathna Deepam*	3 (*n* = 6 in each group)	NM*	I- Control	10.6 ± 0.4	4.2 ± 0.21
						II- Trial drug at the dose of 100 mg/kg of b.wt	13 ± 0.5	6.1 ± 0.2
						III- Trial drug at the dose of 200 mg/kg of b.wt	14.9 ± 0.1	6.7 ± 0.2
						IV- StandardHaematinic syrup	21.8 ± 0.4	6.9 ± 0.1
3	Suba (2014)	*Dhasadeebakkini Chooranam*	*Sigichaa Rathna Deepam*	NM*	14 days	I- Control	10.5 ± 0.85	4.3 ± 0.11
						II- Trial drug at the dose of 200 mg/kg of b.wt	13 ± 0.51	6.5 ± 0.1
						III- Trial drug at the dose of 400 mg/kg of b.wt	15.5 ± 0.22	6.8 ± 0.17
						IV- Standard haematinic syrup	22.1 ± 0.8	7.06 ± 0.1
4	Gnanavel (2013)	*Echuramooli ilai Chooranam*	*Gunapadam Mooligai Vaguppu*	6 (*n* = 6 in each group)	14 days	I- Control	18.4 ± 1.3	4.8 ± 0.3
						II- Disease control	9.05 ± 1.1	4.2 ± 0.2
						III- Trial drug at the dose of 50 mg/kg of b.wt	20.1 ± 0.7	4.8 ± 0.3
						IV- Trial drug at the dose of 100 mg/kg of b.wt	20.1 ± 0.8	4.7 ± 0.2
						V- Trial drug at the dose of 2,000 mg/kg of b.wt	20.4 ± 1.9	4.8 ± 0.2
						VI- Standard haematinic syrup	22.1 ± 1.8	5.1 ± 0.2
5	Ilamathi (2016)	*Karisalankanni chooranam*	*Sigitcharathnadeepam – part II*	4	3 Weeks	I- Control	19.7 ± 0.1	7.3 ± 0.2
						II- Disease control	16.6 ± 1.2	6.0 ± 1.6
						III- Standard haematinic syrup (Vitamin B12)	19.4 ± 2.6	7.0 ± 0.2
						IV- Trial drug at the dose of 400 mg/kg of b.wt	19.8 ± 1.4	7.1 ± 1.8
6	Karpagavalli (2019)	*Karisalankanni chooranam*	NM*	5 (*n* = 5 in each group)	28 days	I- Control	13.4 ± 0.7	6.3 ± 0.1
						II- Trial drug at the dose of 50 mg/kg of b.wt	13.3 ± 0.1	6.4 ± 0.3
						III- Trial drug at the dose of 200 mg/kg of b.wt	13.4 ± 0.1	6.5 ± 0.6
						IV- Trial drug at the dose of 400 mg/kg of b.wt	13.7 ± 0.1	6.6 ± 0.5
7	Padmagreesan (2008)	*Keezhanelli chooranam*	*Koshayi Anuboga vaithiya Bhramma Ragasiyam*	2 (*n* = 5 in each group)	5 weeks	I- Trial drug at the dose of 100 mg/kg of b.wt	9.6 ± 0.1	-
						II- Control	6.1 ± 0.3	-
8	Murugan (2009)	*Pandu seena Usidham*	*NM**	2 (*n* = 5 in each group)	5 weeks	I- Trial drug at the dose of 20 g/kg of b.wt	10.1 ± 0.5	-
						II- Control	5.6 ± 0.4	-
9	Shenbagavalli (2009)	*Madhulai Manappagu*	*NM**	3 (*n* = 3 in each group)	NM	I- Control	5.22 ± 0.21	-
						II- Honey (Vehicle)	6.22 ± 0.29	-
						III- Trial drug at the dose 100 mg	9.2 ± 0.22	-

Continued

S.No	Reference	Trial drug	Siddha literature	No. of groups Intervention period		Groups- intervention type and dosing	After treatment	
							Hb (g/dl) (Mean ± SD)	RBC (×l0^6/ul) (Mean ± SD)
10	Meenakshi Sundaram (2018)	*Madhulai Manappagu*	*NM**	4 (*n* = 6 in each group)	28 days	I- Control	13.5 ± 1.4	8.0 ± 0.9
						II- Disease control	8.6 ± 0.6	5.6 ± 0.5
						III- Standard haematinic syrup in the dose of 10 mg/kg b.wt	11.7 ± 0 .6	8.1 ± 0.3
						IV- Trial drug at the dose of 800 mg/kg of b.wt	11.8 ± 0.8	7.8 ± 0.8
11	Jaseema Parveen (2013)	*Neermulli Chooranam*	*Gunapadam Mooligai Vaguppu*	2 (*n* = 5 in each group)	5 weeks	I- Trial drug at the dose of 1 /100 g b.wt	9.6 ± 0.18	-
						II- Control	5.4 ± 0.12	-
12	Mahalakshmi (2008)	*Saaranaiver chooranam*	*NM**	3 (*n* = 6 in each group)	15 days	I- Control	9.5 ± 1.6	3.1 ± 0.5
						II- Standard haematinic drug (fefol capsules)	12.4 ± 1.5	4.1 ± 0.5
						III- Trial drug at the dose of 500 mg/kg b.wt	13.5 ± 0.8	6.5 ± 0.9
13	Sociya Parvin (2019)	*Siru Vilvathi Elagam*	*Anuboga vaithya navaneetham*	4 (*n* = 6 in each group)	14 days	I- Control	11.0 ± 0.9	8.9 ± 0.2
						II- Disease control	8.02 ± 0.1	5 ± 0.03
						III- Trial drug at the dose of 200 mg/kg of b.wt	9.01 ± 0.2	6.01 ± 2
						IV- Trial drug at the dose of 400 mg/kg of b.wt	10.2 ± 0.2	6.1 ± 2.1
14	Malliga (2008)	*Sennayuruvi Chooranam*	*Gunapadam Mooligai Vaguppu*	2 (*n* = 6 in each group)	NM	I- Control	9.1 ± 1.2	3.1 ± 0.3
						II- Trial drug at the dose of 500 mg/kg of b.wt	13.7 ± 0.2	4.6 ± 0.3
15	Revathi (2012)	*Thaleesa Mathirai*	*Anubava vaithiya murai*	2 (*n* = 6 in each group)	28 days	I- Control	5.8 ± 0.3	-
						II- Trial drug (Dose- NM*)	10.2 ± 0.4	-
16	Asvini (2008)	*Thaetran Karpam*	*Agathiar Attavanai Vagadam*	3 (*n* = 6 in each group)	28 days	I- Control	8.5 ± 2.0	2.8 ± 0.6
						II- Standard haematinic drug (fefol capsules)	13.5 ± 0.8	6.5 ± 0.9
						III- Trial drug at the dose of 500 mg/kg of b.wt	10.6 ± 0.9	3.5 ± 0.3
17	Shanmuga Priya (2017)	*Thiratchai Chooranam*	*Agasthiar Paripooranam 400*	2 (*n* = 3 in each group)	6 weeks	I- Control	6.3 ± 0.2	-
						II- Trial drug at the dose of 100 mg/Kg of b.wt	8.4 ± 0.15	-
18	Balamurugan (2013)	*Tirudharatchatha Chooranam*	*NM**	2 (*n* = 3 in each group)	14 days	I- Control	12.1 ± 0.3	5.2 ± 0.3
						II- Trial drug at the dose 180 mg/kg of b.wt	13.2 ± 0.2	6.2 ± 0.5
19	Sathya Maheswari (2013)	*Valendrapola Chooranam*	*Gunapadam mooligai vaguppu*	6 (*n* = 6 in each group)	14 days	I- Control	16.1 ± 1.4	5.2 ± 0.2
						II- Disease control	9.7 ± 1.1	3.2 ± 0.5
						III- Trial drug at the dose of 100 mg/Kg of b.wt	17.1 ± 0.6	4 ± 0.3
						III- Trial drug at the dose of 200 mg/Kg of b.wt	17.1 ± 0.5	4.8 ± 0.3
						III- Trial drug at the dose of 250 mg/Kg of b.wt	18.5 ± 0.5	4.3 ± 0.2
						IV- Trial drug at the dose of 500 mg/Kg of b.wt	20.6 ± 1.3	5.2 ± 0.5

Continued

S.No	Reference	Trial drug	Siddha literature	No. of groups Intervention period		Groups- intervention type and dosing	After treatment	
							Hb (g/dl) (Mean ± SD)	RBC (×10^6/ul) (Mean ± SD)
Preclinical pharmacological (Efficacy) studies -herbo-mineral/herbo-metal based formulations (*n* = 14)								
1	Kavitha (2018)	*Annabethi Chendhuram*	*Gunapadam Thathu Jeeva Vaguppu*	NM	28 days	I- Control	12.4 ± 1.5	-
						II- Trial drug at the dose of 25 mg/kg of b.wt	13.2 ± 1.3	-
						III- Trial drug at the dose of 125 mg/kg of b.wt	13.9 ± 1.3	-
						IV- Trial drug at the dose of 250 mg/kg of b.wt	13.3 ± 1.1	-
2	Padmagreesan (2008)	*Annabethi Chendhuram*	*Gunapadam II and III*	2 (*n* = 5 in each group)	5 weeks	I-Trial drug at the dose of 20 mg/100 g of b.wt	10.8 ± 0.1	-
						II- Control	6.2 ± 0.1	-
3	Kanmani (2013)	*Aya Chenduram*	*Kannusamiyam vaithyiasegaram*	2 (*n* = 5 in each group)	5 weeks	I-Trial drug at the dose of 20 mg/100 g of b.wt	11.2 ± 0.4	-
						II- Control	5.5 ± 0.28	-
4	Vijayakumar (2015)	*Ayabringaraja Paanidham*	*Gunapadam – Thathu, Jeeva vaguppu*	3 (*n* = 6 in each group)	28 days	I- Control	14.1 ± 0.2	4.3 ± 0.01
						II- Trial drug at the dose of 100 mg/Kg of b.wt	14.2 ± 0.1	4.3 ± 0.1
						III-Trial drug at the dose of 500 mg/Kg of b.wt	15.3 ± 0.2	4.6 ± 0.1
5	Selva Deepa (2009)	*Ayabringa Raja Karpam*	*Siddha Vaidhya Thirattu*	2 (*n* = 5 in each group)	5 weeks	I- Trial drug at the dose of 100 mg/Kg of b.wt	11.2 ± 0.2	-
						II- Control	6.24 ± 0.3	-
6	Kalaivani (2018)	*Ayapodi Elagam*	*NM**	4 (*n* = 10 in each group)	28 days	I- Control	12.6 ± 1.9	-
						II- Trial drug at the dose of 450 mg/Kg of b.wt	13.4 ± 2	-
						II- Trial drug at the dose of 900 mg/Kg of b.wt	13.4 ± 1.5	-
						II- Trial drug at the dose of 1,800 mg/Kg of b.wt	13.5 ± 2.3	-
7	Punitha Lakshmi (2009)	*Gandaga Chenduram*	*Bogar Elunooru*	2 (*n* = 5 in each group)	5 weeks	I-Trial drug at the dose of 20 mg/100g of b.wt	11.4 ± 0.2	-
						II- Control	5.3 ± 0.3	-
8	Jamuna Rani (2013)	*Kaandha Chendhuram*	*Kannusamy Parambarai Vaithiyam*	2 (*n* = 5 in each group)	5 weeks	I- Trial drug at the dose of 20 mg/100 g of b.wt	9.4 ± 0.2	-
						II- Control	5.4 ± 0.2	-
9	Jeevaraj (2018)	*Mandoora chendooram*	*NM**	3 (*n* = 3 in each group)	28 days	I- Control	8.1 ± 2.2	6.0 ± 0.8
						II- Trial drug at the dose of 30 mg/Kg of b.wt	13.0 ± 1.5	6.4 ± 1.7
						III-Trial drug at the dose of 2,000 mg/Kg of b.wt	13.4 ± 1.0	6.7 ± 0.8
10	Nandhini (2019)	*Mandoora Vadagam*	*Sarabendira Vaithiya Muraigal*	5 (*n* = 6 in each group)	21 days	I- Control	12.0 ± 0.3	7.3 ± 0.1
						II- Disease control	7 ± 0.1	4.6 ± 0.4
						III- Standard haematinic syrup	10.5 ± 0.3	6.3 ± 0.1
						IV- Trial drug at the dose of 200 mg/Kg of b.wt	8.4 ± 0.1	5.6 ± 0.1
						V- Trial drug at the dose of 400 mg/Kg of b.wt	10.0 ± 0.1	6 ± 0.6
11	Kannabiran (2012)	*Nimilai Chendhuram*	*Agathiyar Vaithiya Chenduram*	2 (*n* = 6 in each group)	28 days	Group I served as control	11.08 ± 0.34	5.2 ± 0.3
						Group II received trial drug at 28.5 mg/kg of b.wt	14.52 ± 0.20	7 ± 0.13

Continued

S.No	Reference	Trial drug	Siddha literature	No. of groups Intervention period		Groups- intervention type and dosing	After treatment	
							Hb (g/dl) (Mean ± SD)	RBC (×l0^6/ul) (Mean ± SD)
12	Maheswari (2019)	*Thiripalai Mathirai*	*Kadukkai Vallaraiyin Thani Maanbu*	5 (n = 6 in each group)	14 days	I- Control	8.2 ± 0.8	4.1 ± 0.2
						II- Trial drug at the dose of 50 mg/Kg of b.wt	12.3 ± 1.2	4.4 ± 0.5
						III- Trial drug at the dose of 100 mg/Kg of b.wt	12.5 ± 1.2	4.5 ± 0.6
						IV- Trial drug at the dose of 200 mg/Kg of b.wt	13.9 ± 1.3	4.3 ± 0.2
						V- Trial drug at the dose of 400 mg/kg of b.wt	14.2 ± 1.3	4.7 ± 0.7
13	Thiyagarajan (2009)	*Thiriloga Chendhuram*	*Siddha formulary of India*	2 (n = 5 in each group)	5 weeks	I- Trial drug at the dose of 100 mg/Kg of b.wt	10.1 ± 0.8	-
						II- Control	5.7 ± 0.5	-
14	Abdul Abbas (2013)	*Thiripala Mathirai*	*Kadukkai Vallaraiyin Thani Maanbu*	2 (n = 6 in each group)	28 days	I- Control	5.4 ± 0.2	-
						II- Trial drug at the dose of 100 mg/Kg of b.wt	10.7 ± 0.14	-

*NM- Not mentioned, b.wt- Body weight.

heavy metals in their respective trial drugs. 5 studies used ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) and 4 studies used AAS (Atomic Absorption Spectrometry) to analyze and report the detection limits of heavy metals in the trial drugs. Among these 9 preclinical studies, 3 trial drugs namely *Annabedhi Chedhuram* (221. 320 mg/dl), *Mandoora Chendhuram* (812.428 mg/l) and *Ayapodi Ilagam* (12.94%) has greater Iron content then the other trial drugs (Table 4). The other heavy metals like lead, mercury, Cadmium and arsenic were below the detection limits in all the Siddha drugs studied.

Risk of bias assessment

Funnel plot is generated using revman-5.2 to assess publication bias in selected studies. Funnel plot is symmetrical; hence no significant publication bias was observed in this study (Fig. 4).

Meta-analysis

In this meta-analysis, 28 articles evaluating pre-clinical therapeutic effectiveness of Siddha formulations for treating anaemia were included. Quantitative tests for heterogeneity was 95% (I^2 = 95%) for the anaemia related outcomes viz. Hb level and Total RBC and thus suggests there was study variability i.e. heterogeneity; which means significant differences across studies. Hence, random effect model was employed for both the outcomes.

Hb levels before and after treatment

Increase in mean Hb of the trial group which received various Siddha formulations before and after treatment was found to be significant. (SMD: 7, 95% CI: 5.43 to 8.57, n = 28 studies; 153 experimental animals each in control and experimental group; Z Value = 8.75; p Value < 0.00001) as shown in Figure 5.

The mean difference in Hb levels of experimental animals which received Siddha polyherbal formulations and Siddha herbo-metallic/herbo-mineral formulations was also observed. Among these two groups, animals which received Herbo-mineral/Herbo-metallic formulations showed significant increase in Hb levels than Polyherbal formulations [Polyherbal formulations- SMD: 7.61, 95% CI: 5.89 to 9.33, n = 15 studies; 85 experimental animals each in control and experimental group; Z Value = 8.65; p Value < 0.00001; Herbo-mineral/Herbo-metallic formulations- SMD: 10.97, 95% CI: 7.27 to 14.68, n = 13 studies; 76 experimental animals each in control and experimental group; Z value = 5.80; p value < 0.00001] and the same was depicted in Figures 6 and 7 respectively. The difference in Hb levels before and after treatment with the trial drugs have been calculated and it was mentioned in Table 3. About 0.4 to 11.4 g/dl improvement in Hb were observed in these pre-clinical studies after treating with the respective trial drugs. The difference in mean Hb between polyherbal and herbo-mineral/ herbo-metallic formulations was shown in Figure 8.

RBC level before and after treatment

Among 28 studies included, only 14 studies have studied the Total RBC count which is another anaemia related outcome of this meta- analysis. These studies have observed the differences in total RBCs and other blood cell indices between trial and control groups. On quantifying the data, there was a notable improvement in mean RBC before and after treatment. (SMD: 2.40, 95% CI: 1.46 to 3.33, n = 14 studies; 163 experimental animals; Z Value = 5.02; p Value < 0.00001) as shown in Figure 9. Besides, there was no appreciable difference was observed between Polyherbal and herbo-mineral/ herbo-metallic formulations study groups due to negligible amount of studies under each groups.

DISCUSSION

On analyzing the data, neither the poly herbal formulations nor any metal or mineral based herbal formulations produced any toxicological behavioral changes, physiological changes,

Table 2. Detailed description and characterization of included pre-clinical toxicological studies.

Preclinical toxicological evaluation of Siddha formulations used in the treatment of Veluppu noi								
S. No	**Author name**	**Trial drug**	**Reference**	**Acute toxicity study**	**Observed period**	**Sub-acute toxicity/repeated oral toxicity study**	**Observed period**	**Study outcome**
Poly herbal formulations (n = 16)								
1	Kalaiselvi (2017)	*Chitramutti Nei*	NM*	Single dosage of the drug 2,000 mg/kg of b.wt was administered orally to albino mice	14 days	Animals were divided into 3 groups of 6 animals each. Group I was kept as control, while Group II and III were administered with trial drugs at the dose of 200, 400 mg/kg respectively	28 days	No signs of acute and chronic toxicity.
2	Priyadharshini (2012)	*Dhiratchai Chooranam*	*Agasthiyar Paripooranam 400*	Acute toxicity was carried out in Swiss albino mice (n = 20) with a single exposure of 10 times of the recommended therapeutic dose of trial drug (750 mg)	14 days	The trial drug was administered to animals at dose levels of 1× therapeutic dose (13.5 mg/animal), 5× therapeutic dose (67.5 mg/ animal) and10× therapeutic dose (135 mg/ animal).The control animals were administered vehicle only	90 days	No significant toxic effects seen in both acute and repeated oral toxicity.
3	Gnanavel (2013)	*Echuramooli Ilai Chooranam*	*Gunapadam Mooligai Vaguppu*	Single dosage of the drug 2,000 mg/kg of b.wt was administered orally to albino mice	14 days	-	-	No signs of acute toxicity
4	Velayudam *et al.* (2013)	*Kadukkai Mathirai*	Hospital Pharmacopoeia	Single oral dose of trial drug 2,000 mg/kg was administered	14 days	Animals were divided into 4 groups of six animals in each (3 males and 3 females), trial drug was administered to animals three doses 3×, 5×, 10× (36, 180, 360 mg/kg)		No mortality or signs of toxicity observed.
5	Ilamathi (2016)	*Karisalankanni Chooranam*	*Sigitcharathnadeepam – part II*	6 Wistar albino rats were selected and divided into two groups randomly. Trial group received Single dose (2 g/kg)of trial drug orally, Group 2 served as control.	14 days	-	-	No acute toxic effects upto the dose of 2 g/kg
6	Gnanavel (2013)	*Karisalankanni Chooranam*	*Sigicha Rathna Deepam- Part I*	Single dosage of the drug 36 mg/animal [10×] was administered orally to Swiss albino mice	14 days	Wistar albino rats were equally divided into 3 groups administered with 1× dose [36 mg/animal], 5× dose [180 mg/animal] and 10× dose [360 mg/animal] orally once per day	90 days	No obvious changes in behavioural pattern, Body weight, histopathological changes and mortality seen in both acute and repeated oral toxicity study.
7	Karpagavalli (2019)	*Karisalankanni Chooranam*	NM*	Highest dose of 2,000 mg/kg/p.o was used in the acute toxicity study in Wistar albino rats	14 days	48 animals were equally divided into 4 groups .Group I was kept as control, Group II received low dose of trial drug 1× (50 mg), Group III received Mid dose 4× (200 mg) of trial drug and Group IV received High dose 8× (400 mg) of trial drug	28 days	The acute toxicity study of the trial drug did not exhibit any significant toxicity at the dose of 2,000 mg /kg body weight. Sub-acute toxicity study did not exhibit any significant toxic effects upto the dose of 400 mg/kg.

Continued

Preclinical toxicological evaluation of Siddha formulations used in the treatment of Veluppu noi								
S. No	**Author name**	**Trial drug**	**Reference**	**Acute toxicity study**	**Observed period**	**Sub-acute toxicity/repeated oral toxicity study**	**Observed period**	**Study outcome**
Poly herbal formulations (*n* = 16)								
8	Meenakshi Sundaram (2018)	*Madhulai Manappagu*	NM*	The trial drug was orally administered at a dose of 5,000 mg/kg bodyweight to the Wister Albino rats	14 days	In the 28 days(sub-acute) and 90 days(chronic) repeated oral toxicity study, the animals were grouped into 4 groups and the trial drug was administered continuously for a period of 28 and 90 days in the doses of 500, 750 and 1,000 mg/kg/day to 3 groups and one group was maintained as a control	28 days/ 90 days	No obvious toxic effects seen in acute, sub-acute toxicity and repeated oral toxicity study.
9	Jaseema Parveen (2013)	*Neermulli chooranam*	*Gunapadam Mooligai Vaguppu*	10 albino rats were equally divided into 5 groups, Group I- V received trial drug at the dose of 100, 200, 400, 800 mg, 1,600 mg/kg. of b.wt respectively.	14 days	-	-	No mortality and any remarkable pathological findings seen.
10	Arunmozhi (2013)	*Puli ilai Chooranam*	NM*	Highest dose of 2,000 mg/kg/p.o was used in the acute toxicity study in Wistar albino rats	14 days	-	-	No signs of toxicity seen
11	Mahalakshmi (2008)	*Saaranaiver Chooranam*	NM*	Highest dose of 2,000 mg/kg/p.o was used in the acute toxicity study	14 days	-	-	No drug related mortality and morbidity seen
12	Malliga (2008)	*Sennaiyuruvi Chooranam*	*Gunapadam Mooligai Vaguppu*	The highest dose of 2,000 mg/kg/p.o was used in the acute toxicity study.	14 days	-	-	No signs of toxicity seen
13	Sociya Parvin (2019)	*Siru Vilvathi Elagam*	*Anuboga vaithya navaneetham*	Anaemia induced rats were divided into 5 groups and were administered single time at the dose of 5, 50, 300, 1,000 and 2,000 mg/kg	14 days	The rats were divided into 4 groups. Group I was kept as control, while Group II, III and IV were administered with trial drugs at the dose of 200, 400 and 600 mg/kg respectively	28 days	No obvious toxic effects seen even at the dose of 2,000 mg/ Kg in acute toxicity studies and also no signs of toxicity at the dose of 600 mg/kg were observed in sub-acute toxicity studies.
14	Asvini (2008)	*Thaetran Karpam*	*Agathiar Attavanai Vagadam*	20 swiss albino mice received single dose of trial drug (2,000 mg/kg)	14 days	-	-	No significant toxic effects seen in acute oral toxicity study.
15	Balamurugan (2013)	*Tirudharatchatha Chooranam*	*Agasthiyar vaithiya rathinachurukkam*	Highest dose of 2,000 mg/kg/p.o was used in the acute toxicity study in Wistar albino rats	14 days	In Sub-acute toxicity study, The rats were divided into 2 groups. Group I kept as control, while group II received aqueous suspension of trial drug	28 days	No obvious toxic effects seen in both acute and sub-acute toxicity

Continued

Preclinical toxicological evaluation of Siddha formulations used in the treatment of Veluppu noi								
S. No	**Author name**	**Trial drug**	**Reference**	**Acute toxicity study**	**Observed period**	**Sub-acute toxicity/repeated oral toxicity study**	**Observed period**	**Study outcome**
Poly herbal formulations (*n* = 16)								
16	Sathya Maheswari (2013)	*Valendraphola Chooranam*	*Gunapadam mooligai vaguppu*	The trial drug was administered maximum upto the dose of 5g/kg	14 days	24 rats of either sex were divided into 4 groups (*n* = 6 in each group). Group I served as control, while groups II, III and IV were administered daily with the trial drug for at the dose of 0.1, 0.25 and 0.5 g/kg respectively.	28 days	In the acute toxicity study at the dose level of 5 g/kg moderate toxic symptoms like alertness, grooming, touch response, writhing and hypnosis were observed, No toxic effect was observed upto 500 mg/kg in sub-acute toxicity.
Preclinical toxicity evaluation - Herbo-mineral/herbo-metal based formulations (*n* = 14)								
1	Kavitha (2018)	*Annabethi Chendhuram*	*Gunapadam Thathu Jeeva Vaguppu*	20 albino rats were equally divided into 2 groups, Group 1 was kept as control and Group II was administered with the trial drug in the dose of 250 mg/kg.	14 days	Long-term toxicity study was conducted in 3 doses low dose (25 mg/kg b.wt), mid dose(125 mg/kg b.wt), high dose (250 mg/kg b.wt).	90 days	No obvious toxic effects seen in both acute and repeated oral toxicity upto the dose of 250 mg/kg body weight of animal.
2	Kanmani (2013)	*Aya Chenduram*	*Kannusamiyam vaithyiasegaram*	Rats were divided into 5 groups (*n* = 2 in each group), 1 group is kept as control group, Other groups from II-V were administered with the trial drug at the doses of 40, 80, 160, 320 mg/kg respectively.	14 days	-	-	No toxic effects seen upto the dose of 320 mg/kg of trial drug.
3	Vijayakumar (2015)	*Ayabringaraja Paanidham*	*Gunapadam – Thathu, Jeeva vaguppu*	Female albino mice were divided into 2 groups (*n* = 5 in each group), Group 1 kept as control, Group 2 animals administered the trial drug at the dose of 2,600 mg/kg (10 times higher the therapeutic dose)	14 days	48 wistar rats divided into 4 equal groups, Group 1 served as control, while Groups II, III and IV served as trial animals with varied doses.	28 days	No toxic effects seen in results of acute and sub-acute toxicity studies.
4	Kalaivani (2018)	*Ayapodi Elagam*	*Anubogavaithiya Navaneetham*	40 Wistar albino rats were divided into 2 equal groups and administered with saline(Group-I, Control), Trial drug (5,000 mg/kg) as single dose	14 days	The animals in both sex were divided in four groups (*n* = 10 in each group). Group-I served as control and the other three groups II, III and IV for test drug of Low dose (450 mg/kg/b.wt), mid dose(900 mg/kg/b.wt) and high dose(1,800 mg/kg b.wt) respectively	90 days	No obvious changes in behavioural pattern, Body weight, histopathological changes and mortality seen in both acute and repeated oral toxicity.

Continued

Preclinical toxicological evaluation of Siddha formulations used in the treatment of Veluppu noi								
S. No	**Author name**	**Trial drug**	**Reference**	**Acute toxicity study**	**Observed period**	**Sub-acute toxicity/repeated oral toxicity study**	**Observed period**	**Study outcome**
Poly herbal formulations (*n* = 16)								
5	Punitha Lakshmi (2009)	*Gandaga Chenduram*	*Bogar Elunooru*	10 Anaemia induced rats which were divided into 5 equal groups and received the trial medicine in different doses from 100 mg/ animal up to 1,600 mg/ animal.	14 days	-	-	No acute toxic effects upto the dose of 1,600 mg/animal.
6	Benitta (2008)	*Kantha Chenduram*	*Gunapadam II and III*	30 wistar albino rats were selected and divided into 6 groups, each group administered with the trial *drug kantha chenduram* in different graded dosages maximum up to 1,600 mg/animal by enteral route.	14 days	15 wistar albino rats were selected and divided into 3 groups. The first group kept as control (water), while second and third group were administered with *kantha chenduram* at the dose of 100 mg/animal and 200 mg/animal respectively.	28 days	No acute toxicity and subacute toxic effects seen
7	Jamuna Rani (2013)	*Kaandha Chenduram*	NM*	10 rats were divided into 5 groups each consisting of 2 rats, 1 group is kept as control group and other groups were given the trial drug at the doses of 40/80/160/320 mg/kg of b.wt	14 days	-	-	No obvious toxic effects seen up to the dose of 320 mg/kg of b.wt
8	Jeevaraj (2018)	*Mandoora Chendooram*	NM*	Animals were divided into 3 groups, (*n* = 3 in each group), group 1-control, and the other 2 groups II and III were treated with test drug at two different doses 300, 2,000 mg/kg. B.wt respectively.	14 days	The animals in both sexes were divided in 4 groups (*n* = 6 in each group) group – I served as control and the other 3 groups (II, III and IV) were treated as test group low dose – 2.34 mg/kg. b.wt), mid dose – 1.7 mg/kg. b.wt), high dose – 23.4 mg/kg. b.wt) respectively	28 days	No acute toxicity and subacute toxic effects seen
9	Nandhini (2019)	*Mandoora Vadagam*	*Sarabendira Vaithiya Muraigal*	Female wistar rats in controlled age and body weight were selected and divided equally into 5 groups. The trial drug was administered orally at 5, 10, 300, 1,000, 2,000 mg/kg body weight of animal as suspension along with water	14 days	40 albino rats were divided equally in 4 groups and administered the trial drug suspended with water at the dose of 200, 400, and 600 mg/ kg respectively from group II-IV. Group 1 served as control.	28 days	The acute toxicity study shows that *mandoora vadagam* did not produce any toxic effect even at the dose of 2,000 mg/kg to rats. In sub-acute toxicity study also, the trial drug was considered as safe at all doses.

Continued

Preclinical toxicological evaluation of Siddha formulations used in the treatment of Veluppu noi								
S. No	**Author name**	**Trial drug**	**Reference**	**Acute toxicity study**	**Observed period**	**Sub-acute toxicity/repeated oral toxicity study**	**Observed period**	**Study outcome**
Poly herbal formulations (*n* = 16)								
10	Kannabiran (2012)	*Nimilai Chendhuram*	*Agathiyar Vaithiya Chenduram*	Single gavage of 28.5 mg/kg of b.wt was given to a group of 6 animals	14 days	The animals were divided into 2 groups of each with six animals. Group I served as control; group II received aqueous suspension of *nimilai chenduram* (nc) 28.5 mg/kg of b.wt	28 days	Trial drug evaluated in this study has no toxicity and safe to use.
11	Janet Sheeba (2012)	*Siddha Mandooram*	*Gunapadam II and III*	20 swiss albino mice received single dose of *siddha mandooram* (4,000 mg/kg)	14 days	40 swiss albino rats which were divided into 4 equal groups administered at the dose of control group (vehicle only), 1× (72 mg/animal), 5× (360 mg/animal), 10× (720 mg/animal) respectively.	90 days	No acute toxicity up to the dose of 4,000 mg/kg and no repeated oral toxic effects up to the dose of 720 mg/animal.
12	Rajesh *et al.* (2014)	*Thiriloga Chendhuram*	NM*	Animals were divided into 6 groups (*n* = 5 in each group) group I served as control, group II- VI received single gavage of trial drug at the doses of 40 m, 80, 160, 320 mg, 640 mg/kg of b.wt	14 days	15 albino rats were divided into 3 groups. Group I served as control, group II and III received trial drug at the doses of 40 m, 80 mg/kg of b.wt	90 days	Trial drug did not produce any mortality is acute toxicity study at the dose level ranging from 40 mg to 640 mg/body weight of the animal. The chronic toxicity studies revealed that the drug has some harmful effect like mild interstitial oedema with haemorrhage in heart. No remarkable changes in liver/kidney on long term administration.
13	Maheswari (2019)	*Thiripalai Mathirai*	*Kadukkai Vallaraiyin Thani Maanbu*	Anaemia induced mice were treated at the dose up to 2,000 mg/kg	14 days	In sub-acute toxicity, group I animals were treated with normal saline, group II-v received 50, 100, 200 and 400 mg/kg of trial drug.	28 days	The trial drug is considered to be safe in acute and sub-acute toxicity study in animals.
14	Sridevi (2017)	*Veera Aya Chendhuram*	NM*	Animals were divided into 5 groups (*n* = 3 in each group), one group as control and the other four groups were treated with test drug at four different doses (5, 50, 300, 2,000 mg /kg of b.wt)	14 days	The animals were divided in 4 groups (*n* = 10 in each group). Group I served as control, group II, III andIV were administered at the doses of 4.68, 23.4, 46.8 mg respectively	28 days	The trial drug is considered to be safe in acute and sub-acute toxicity study in animals.

NM*- Not mentioned, b.wt- Body weight, P.O-Per oral.

Table 3. Difference in Hb levels before and after treatment with trial drugs.

S.No	Trial drug	Author	Hb levels (g/dl)		Difference/ Increase in Hb (g/dl)
			Before treatment	After treatment	
1	*Thiripala Mathirai*	Abdul Abbas (2013)	5.4	10.7	5.3
2	*Thaetran Karpam*	Asvini (2008)	8.5	10.6	2.1
3	*Tirudharatchatha Chooranam*	Balamurugan (2013)	12.1	13.2	1.1
4	*Echuramooli ilai Chooranam*	Gnanavel (2013)	9	20.4	11.4
5	*Kaandha Chendhuram*	Jamuna Rani (2009)	5.4	9.4	4
6	*Neermulli Chooranam*	Jaseema parvin (2013)	5.4	9.6	4.2
7	*Mandoora Chendhuram*	Jeevaraj (2018)	8.1	13.4	5.3
8	*Chitramutti Nei*	Kalaiselvi (2017)	10.6	14.9	4.3
9	*Ayapodi Elagam*	Kalaivani (2018)	12.6	13.4	0.8
10	*Aya Chendhuram*	Kanmani (2013)	5.5	11.2	5.7
11	*Karisalankanni Chooranam*	Karpagavalli (2019)	13.3	13.7	0.4
12	*Saaranaiver Chooranam*	Mahalakshmi (2008)	9.5	13.5	4
13	*Thiripalai Mathirai*	Maheswari (2019)	8.2	14.2	6
14	*Sennaiyuruvi Chooranam*	Malliga (2008)	9.1	13.7	4.6
15	*Madhulai Manapagu*	Meenakshi Sundaram (2018)	8.6	11.8	3.2
16	*Pandu Seena Usidham*	Murugan (2009)	5.6	10.1	4.5
17	*Mandoora Vadagam*	Nandhini (2019)	7	10.1	3.1
18	*Keezhanelli Chooranam*	Padmagreesan (2008)	6.1	9.6	3.5
19	*Annabedhi Chendhuram*	Padmagreesan (2008)	6.2	10.8	4.6
20	*Gandhaga Chendhuram*	Punitha Lakshmi (2009)	5.3	11.4	6.1
21	*Nimilai Chendhuram*	Kannabiran (2012)	11	14.52	3.52
22	*Thaleesa Mathirai*	Revathi (2012)	5.8	10.2	4.4
23	*Valendrapola Chooranam*	Sathya Maheswari (2013)	9.7	20.6	10.9
24	*Ayabringaraja Karpam*	Selva Deepa (2009)	6.24	11.2	4.96
25	*Thiratchai Chooranam*	Shanmuga Priya (2013)	6.3	8.4	2.1
26	*Siruvilvathi Elagam*	Sociya Parvin (2019)	8.02	9.01	0.99
27	*Thiriloga Chendhuram*	Thiyagarajan (2009)	5.7	10.1	4.4
28	*Ayabringaraja Paanidham*	Vijayakumar (2015)	14.1	15.3	1.2

histological changes, bio-chemical changes and mortality up to the therapeutic dose level. In 10× of the therapeutic dose as single oral gavage, the Siddha formulation "*Valendrapola Chooranam*" showed some moderate toxic symptoms like alertness, increased touch response, writhing and hypnosis. Another drug namely "*Thiriloga Chendhuram*" at 3× therapeutic dose for a period of 90 days, showed some mild interstitial edema with hemorrhage in heart. However, both the drugs were very much safer at therapeutic dose level as no adverse signs or symptoms were observed. This kind of toxicity at higher doses might be due to non-adherence of standard protocols during trial drug preparations.

Metals are toxic in its natural inorganic form. Upon treatment with various polyherbal juices and adopting various techniques like grinding, calcination, etc for herbo-metallic drug preparations, the particle size of the metals would be reduced and reaches nano size which can be easily absorbed under physiological conditions. When a metal remains unprocessed during drug preparation, it leads to accumulation in various major organs and the same might initiate metal induced tissue damage through free radical mechanisms. As Siddhars wrote their experiences in the form of classical literatures, strict adherence to standard protocols during drug preparation, appropriate therapeutic dose, adjuvants used and period of invention were very much essential factors for the usage of herbo-metallic preparations. This might be the reason for the safe nature of the trial drugs at their therapeutic doses and the same implies that the pre-clinical study was done following the appropriate standards and guidelines involved. Though some toxicity were observed at 10× and 3× therapeutic doses of two Siddha formulations, the same may be negligible as that larger doses will never be employed for the treatment of any diseases. Hence it can be established that all the Siddha formulations analyzed in this study was proved to be safe in their respective therapeutic doses.

The presence of heavy metals in a formulation is a highly concerning parameter, regarding safety in human use of the trial drugs. In addition to anaemia related outcomes, heavy

Table 4. Minerals and heavy metals levels in trial drugs.

S.no	Trial drug	Author	Method used	Outcome
1	*Siddha Mandooram*	Janet Sheeba (2012)	ICP-OES	The trial drug contains iron, magnesium, sodium, phosphurus. Other heavy metals were below the detection level
2	*Mandoora Chenduram*	Jeevaraj (2018)	ICP-OES	The trial drug contains large amount of Fe (812.428 mg/l), copper, cobalt. Other heavy metals were below the detection level
3	*Ayapodi Elagam*	Kalaivani (2018)	AAS	The trial drug contains iron-12.94%. Other heavy metals were below the detection level
4	*Aya Chenduram*	Kanmani (2013)	ICP-OES	The trial drug showed the presence of the following elements namely Fe, S, Sb, Zn, Cu, Co, Bi in the descending manner in quantitative and qualitative basis.
5	*Annabethi Chenduram*	Kavitha (2018)	ICP-OES	The presence of heavy metals such as mercury, lead, arsenic, cadmium is below detection limit. Presence of iron is 221.320 mg/ dl, phosphorous-96.327 mg/dl and some other elements are present in trace levels.
6	*Mandoora Vadagam*	Nandhini (2019)	ICP-OES	The drug contains essential elements such as calcium, iron, pottasium, magnesium, sodium, phosphorus. Other heavy metals were below the detection level
7	*Gandhaga Chenduram*	Punitha Lakshmi (2009)	AAS	The trial drug showed the presence of essential metals within permissible limits
8	*Aya Bringaraja Karpam*	Selva Deepa (2009)	AAS	The trial drug showed the presence of essential metals within permissible limits
9	*Thiriloga Chendhuram*	Thiyagarajan (2009)	AAS	The heavy metals and trace elements like Lead, Mercury, Cadmium, Arsenic were present in the drug within the normal WHO limits.

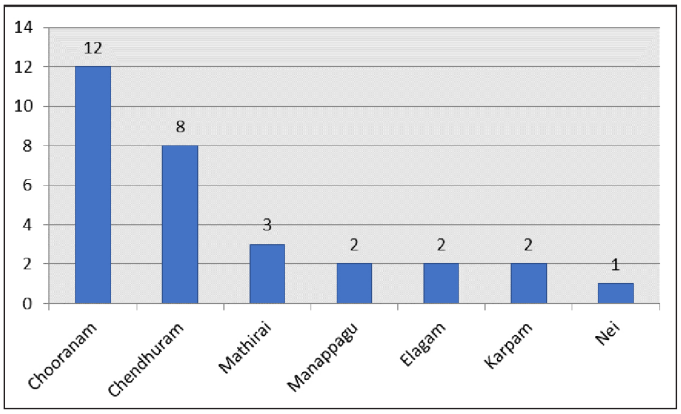

Figure 2. Frequency of type of medicines used in pre-clinical pharmacological analysis.

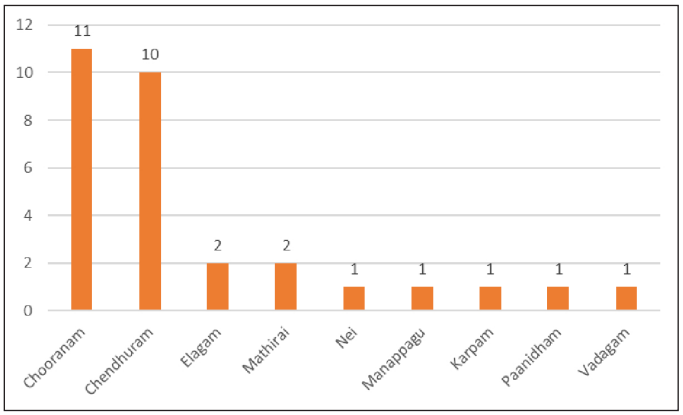

Figure 3. Frequency of type of medicines used in pre-clinical toxicological analysis.

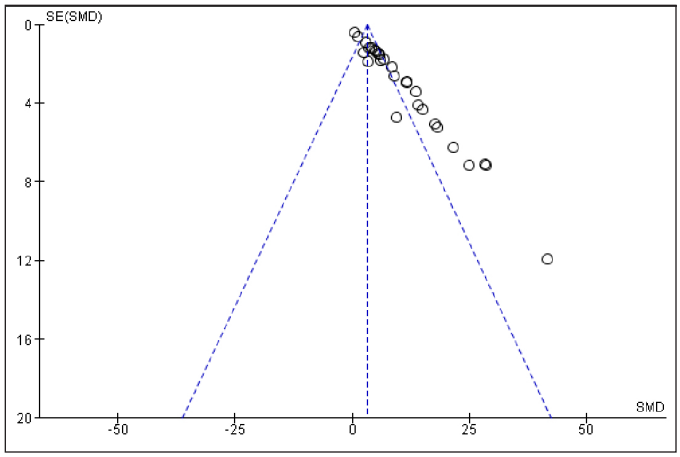

Figure 4. Funnel plot of included studies.

metal analysis of many herbo-mineral and herbo-metallic drugs carried out by the included studies were also observed in this analysis. The herbo-mineral and herbo-metallic trial drugs such as *Siddha Mandooram, Mandoora Chenduram, Ayapodi Elagam, Aya Chenduram, Annabethi Chenduram, Mandoora Vadagam, Gandhaga Chenduram, Aya Bringaraja Karpam* were tested for heavy metals in their respective trials. In all the trials, concentration of heavy metals like Lead, Cadmium, Mercury and Arsenic were found to be below the detection limit. This result ensures the safe usage of the herbo-mineral and herbo-metallic formulations even for a longer treatment period.

SUMMARY OF FINDINGS

In analyzing the results, in terms of subjective and hematological indicators, most of the Siddha formulations

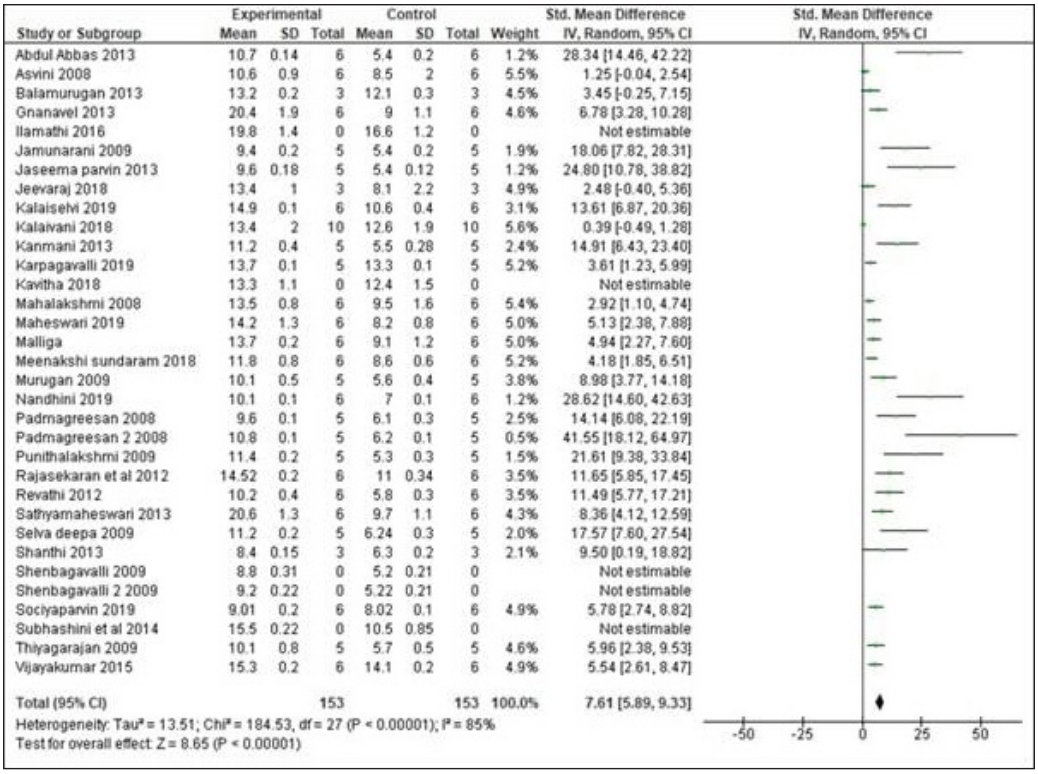

Figure 5. Forest plot showing Hb difference before and after treatment.

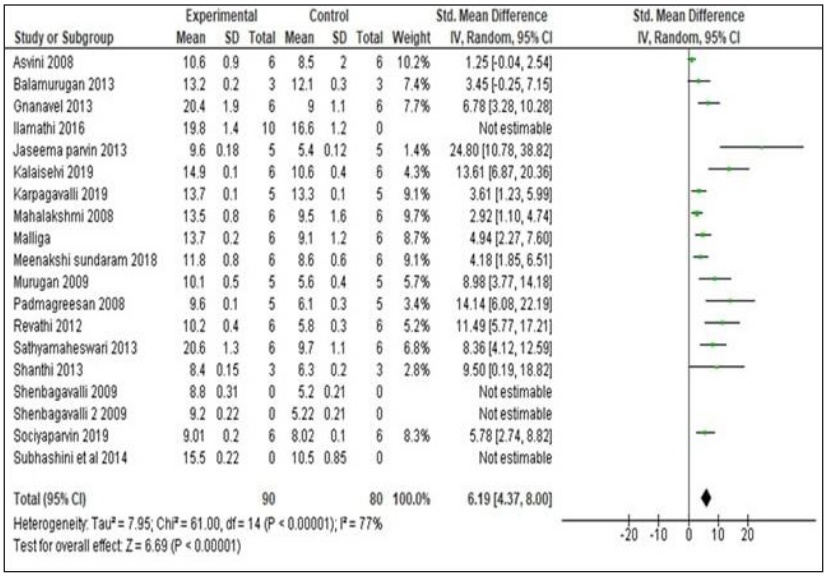

Figure 6. Forest plot depicting Hb difference in polyherbal formulations.

showed statistically significant results and hence proved to be effective in correcting anaemia. *Echuramooli Ilai Chooranam* in polyherbal formulations, *Gandhaga Chendhuram* in Herbo-metallic formulations and *Thiripalai Mathirai* in Herbo-mineral formulations showed highest increase in Hb and RBC. Summary of pre-clinical pharmacological, pre-clinical toxicological studies and clinical studies carried out in Siddha formulations are descripted in Table 5. The Siddha formulations in which all the pre-clinical and clinical trials have been done can be used for therapeutic usage. However, studies with larger sample size and RCT's should be conducted to ensure its reliability.

LIMITATIONS

As most of the studies did not mention the levels of other anaemia related outcomes like serum Iron, Ferritin, Transferrin, total iron binding capacity, etc., the efficacy of these trial drugs could not be strongly validated. The heterogeneity of this meta-analysis is very high which could be attributable to varied sample

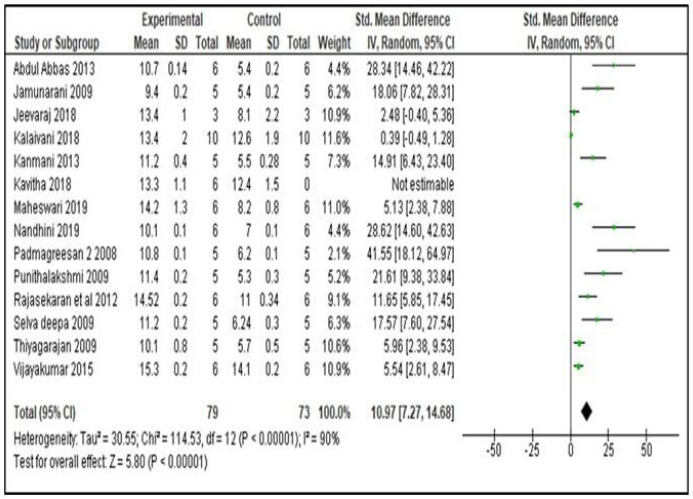

Figure 7. Forest plot depicting Hb difference in herbo-metallic/herbo-mineral formulations.

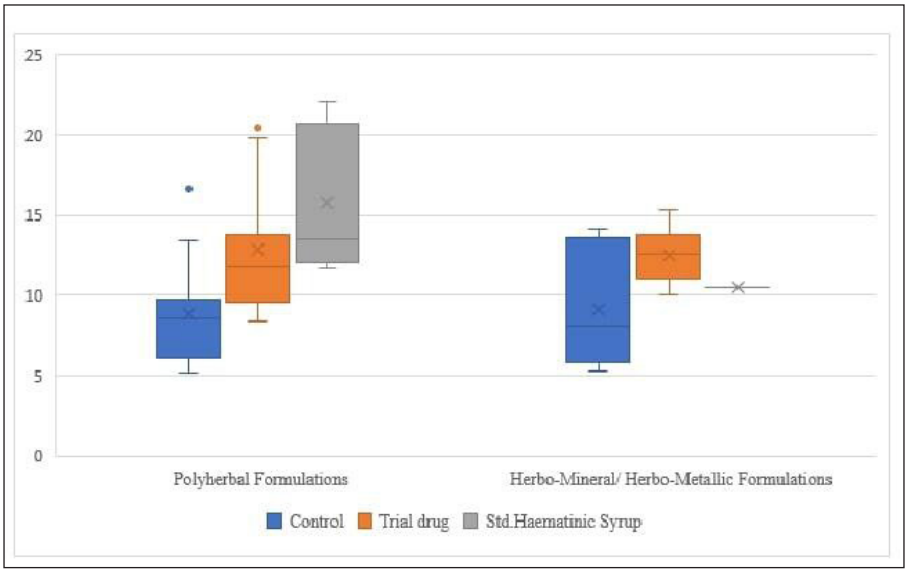

Figure 8. Box plot showing mean increase in Hb in pre-clinical pharmacological studies.

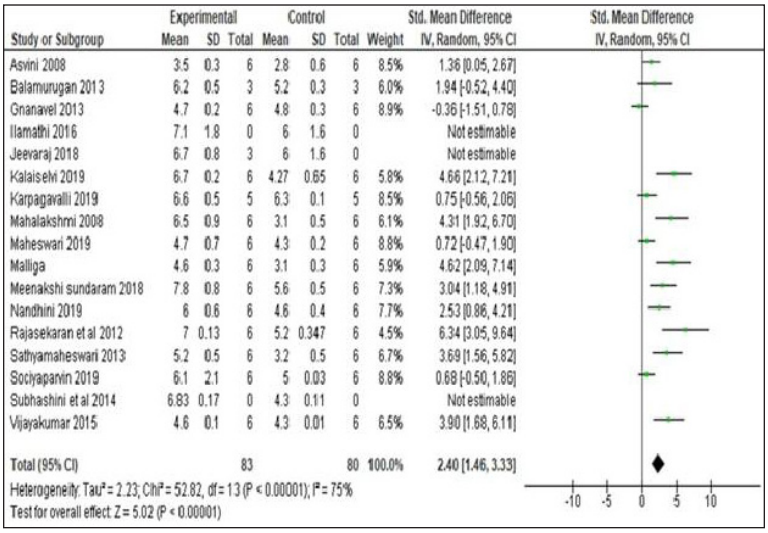

Figure 9. Forest plot showing total RBC count—before and after treatment.

Table 5. Summary of pre-clinical and clinical studies conducted in Siddha formulations for anaemia.

S.No	Trial Drug with Reference	Pre-clinical Pharmaological (Efficacy) studies	Preclinical Toxicological studies	Clinical Studies
1	*Annabedhi Chendhuram*	Yes	Yes	Yes
2	*Aya Bringaraja Karpam*	Yes	No	Yes
3	*Aya Chenduram*	Yes	Yes	Yes
4	*Ayabringaraja Paanidham*	Yes	Yes	Yes
5	*Ayapodi Ilagam*	Yes	Yes	No
6	*Bringaraja Chooranam*	Yes	No	No
7	*Chitramutti Nei*	Yes	Yes	Yes
8	*Dhasadeepakkini Chooranam*	Yes	No	No
9	*Echuramooli Ilai Chooranam*	Yes	Yes	Yes
10	*Gandaga Chenduram*	Yes	Yes	Yes
11	*Kaandha Chendhuram*	Yes	Yes	Yes
12	*Kadukkai Mathirai*	No	Yes	No
13	*Kantha Chendhuram*	No	Yes	No
14	*Karisalankanni Chooranam*	Yes	Yes	Yes
15	*Keezhanelli Chooranam*	Yes	No	Yes
16	*Madhulai Manappagu*	Yes	Yes	Yes
17	*Mandoora Chenduram*	Yes	Yes	No
18	*Mandoora Vadagam*	Yes	Yes	No
19	*Neermulli Chooranam*	Yes	Yes	Yes
20	*Nimilai Chendhuram*	Yes	Yes	No
21	*Pandu Noikku Kalappu Thool*	No	No	Yes
22	*Pandu Seena Usidham*	Yes	No	Yes
23	*Puli Ilai Chooranam*	No	Yes	Yes
24	*Saaranaiver Chooranam*	Yes	Yes	No
25	*Sarakkondrai Chooranam*	No	No	Yes
26	*Sennayuruvi Chooranam*	Yes	Yes	Yes
27	*Siddha Mandooram*	No	Yes	Yes
28	*Siru Vilvathi Elagam*	Yes	Yes	Yes
29	*Thaetran Karpam*	Yes	Yes	Yes
30	*Thaleesa Mathirai*	Yes	No	Yes
31	*Thiratchai Chooranam*	No	Yes	Yes
32	*Thiriloga Chendhuram*	Yes	Yes	No
33	*Thiripalai Mathirai*	Yes	Yes	Yes
34	*Tiridharatchatha Chooranam*	Yes	Yes	Yes
35	*Valendrapola Chooranam*	Yes	Yes	Yes
36	*Veera Aya Chendhuram*	No	Yes	No

sizes, differences in methodologies, period of intervention and certain limitations in different studies. However, Random effect model was employed in forest plot to address the heterogeneity. Funnel plot showed no publication bias. Further, a greater number of RCTs are needed to provide high level evidences.

CONCLUSION

Since many ages, the therapeutic effectiveness of the Siddha system of medicine in treating a variety of illnesses has been established. However, there are some impediments limiting the propagation of Siddha medicine globally, including a dearth of supporting evidence and a lack of validation. This study, therefore, sought to establish the safety and efficacy of Siddha formulations in the treatment of anaemia through adequate documentation. The present study showcased that all the Siddha formulations proposed to treat anaemia in Siddha literatures were proved its safety and efficacy in animal models. Besides. many of these Siddha formulations were not in use in daily practice to treat anaemia. Hence, this study suggests that Siddha formulations which proved its efficacy in pre-clinical trials may be taken either to clinical trials with larger samples size or a well- designed RCTs to make it more feasible to treat anaemia. This study will further

pave way to prescribe these Siddha formulations in public health initiatives and programs to reduce the incidence and prevalence of anaemia in people of all ages globally.

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AUTHOR CONTRIBUTIONS

All the authors have made significant contributions to the concept, design, acquisition of data, analysis and interpretation; and also in drafting and revisions of this article. All the authors are also eligible to be an author based on the guidelines of International Committee of Medical Journal Editors (ICMJE).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVALS

This study does not involve any animal or human subjects and hence not applicable.

DATA AVAILABILITY

All data collected, generated and analyzed for the study are included within this manuscript.

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