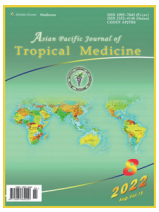

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Efficacy and safety of pharmacotherapeutic interventions used in visceral leishmaniasis clinical trials: A systematic review and network meta−analysis

Aamir Bashir, Gautam Sahu, Ishfaq Rashid, Pramil Tiwari✉

Department of Pharmacy Practice, National Institute of Pharmaceutical Education and Research, S.A.S. Nagar Mohali, 160062, Punjab, India

ABSTRACT

Objective: To compare the efficacy and safety outcomes of different antileishmanial agents used in visceral leishmaniasis clinical trials.

Methods: A systematic literature search in PubMed/MEDLINE, EMBASE, Cochrane, and Google Scholar was done using keywords "randomized controlled trials", "antileishmanial" and "visceral leishmaniasis". The outcomes included were cure rate, overall withdrawals, relapse rate, and treatment-emergent adverse events. Effect estimates through the frequentist network meta-analysis approach were presented as *OR* with 95% *CI*. Rankogram plots were used for identifying the "best intervention" based on p-scores obtained using the surface under the cumulative ranking. The risk of bias was evaluated by using Pedro Scale.

Results: Seventeen randomized controlled trials with 5 143 visceral leishmaniasis patients who received different antileishmanial agents (amphotericin B, miltefosine, paromomycin, meglumine antimoniate, sodium stibogluconate, sitamaquine, and pentavalent antimonials) and met the inclusion criteria were included. For efficacy outcomes of the treatments, the rankogram of the network meta-analysis revealed that paromomycin (p-score=0.814 8) has the highest probability of being best in the pool, followed by sodium stibogluconate (*OR* 0.82, 95% *CI* 0.24-2.79, p-score=0.758 0), amphotericin B+miltefosine (*OR* 0.66, 95% *CI* 0.02-19.04, p-score=0.732 9) as compared to the remaining treatments; however, the most of the treatment-emergent adverse events were reported with sitamaquine.

Conclusions: Paromomycin reported the highest cure rates, while the maximum treatment-emergent adverse events were seen with sitamaquine.

KEYWORDS: Visceral leishmaniasis; Treatment; Efficacy; Safety; Network meta-analysis

1. Introduction

Visceral leishmaniasis (VL) or kala-azar, caused by parasite *Leishmania* (*L.*) *donovani*, is seen as a neglected endemic in Asia, East and North Africa, South America, and Southern Europe[1]. Around 0.2-0.4 million of new cases of VL occur annually worldwide, of which 60% of the cases are reported from India, predominantly from Bihar and West Bengal (northeastern region)[2]. As a vector-borne disease, it is transmitted to humans after a bite of an infected sandfly[3]. In developing countries, poverty is considered as the major underlying cause and threatening factor of this disease[4]. The poor nutritional status, increased morbidity, and faster progression of disease lead to a higher risk of mortality in VL. The disease is fatal if left untreated; however, with advancements in

Significance

Several antileishmanial drugs are currently available to treat visceral leishmaniasis patients. Previously, meta-analyses of individual treatments were done to present their safe and effective use. Meanwhile, no evidence is available on comparative efficacy and safety of recommended therapeutic options. This study for the first time compared all the available interventions and ranked them in a series based on data of cure rates or associated adverse events.

✉To whom correspondence may be addressed. E-mail: ptiwari@niper.ac.in

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both the diagnosis and treatment in recent years, the fatality rate is reduced[5]. Available evidence demonstrates the increasing case fatality or poor prognosis is associated with weight loss, fever, jaundice, splenomegaly, hepatomegaly and/or lymphadenopathies, hemorrhage, and anemia[6].

Earlier, pentavalent antimonial compounds were used as the first-line treatment[7]. However, due to inherent toxicity and frequent parasitic resistance, new treatments evolved over time and showed enhanced therapeutic efficacy and safety against VL. Cases resistant to antimony compounds are preferably treated with amphotericin B, however, the drug is expensive and requires hospitalization and close monitoring for weeks.

Currently, amphotericin B (3 different formulations), miltefosine, paromomycin (PM), meglumine antimoniate (MA), sodium stibogluconate (SSG), sitamaquine, pentavalent antimonials (PA), ketoconazole, fluconazole *etc*. are used individually or in different combinations[8]. The response to any specific treatment protocol depends upon geographical location, parasite species, prescribed dose and the presence of any secondary infection. In a systematic review, Pokharel *et al*. concluded that paromomycin can be a drug of choice for treatment of VL in the Indian subcontinent and in the regions where expensive drugs such as liposomal amphotericin B are not readily available[9]. Similarly, several other results comparing different above-mentioned therapeutic agents were published. However, an effective and safe antileishmanial agent remains an important therapeutic target to treat the patients of visceral leishmaniasis.

Although several meta-analyses were conducted on effectiveness of different individual antileishmanial agents, but none of the studies are found which evaluated the comparative efficacy and safety data between the recommended therapeutic options using a network meta-analysis approach. Further the evidence available is inconsistent across the studies and all these drugs are not evaluated in head-to-head trials for treating VL. Therefore, to fill this evidence gap, this study aimed to summarize all the available data of randomized controlled trials (RCTs) to evaluate the comparative efficacy and safety of antileishmanial agents used in patients with VL. The evidence generated shall help the policymakers to personalize a safe, effective, affordable, and accessible treatment according to the need of patients.

2. Materials and methods

The current review was performed and reported in accordance with the preferred reporting items for systematic reviews and network meta-analyses[10]. A protocol of this study is available in PROSPERO (No. CRD42022308379).

2.1. Search strategy

Relevant RCTs were searched by using following electronic databases from their inception until November 1, 2021: PubMed/MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, and Google Scholar that evaluated the comparative effect of antileishmanial interventions in patients with VL. In addition, a manual search of Clinicaltrials.gov and references of retrieved articles was also performed to identify the relevant articles. Searches were restricted to the English language only. The detailed search strategy used in PubMed and EMBASE is provided in Supplementary Tables S1 and S2.

Table 1. Study characteristics of included randomized clinical trials.

Authors, year	References	Country	Sample size (*n*)	Allocation	Intervention model	Masking	Location	No. of arms	Comparator
Seaman, 1993	[13]	United Kingdom	200	R	PA	OL	SC	2	OCT
Jha, 1998	[14]	India	120	R	PA	OL	SC	4	OMT
Thakur, 2000	[15]	India	150	R	PA	OL	SC	3	OCT
Sundar, 2002	[16]	India	398	R	PA	OL	SC	2	OMT
Sundar, 2004	[17]	India	153	R	PA	OL	SC	3	OMT
Sundar, 2007	[18]	India	667	R	PA	OL	SC	2	OMT
Sundar, 2008	[19]	India	226	R	PA	OL	SC	5	OCT
Sundar, 2010	[20]	India	412	R	PA	OL	SC	2	OMT
Hailu, 2010	[21]	Sudan	405	R	PA	OL	MC	3	OMT
Sundar, 2011	[22]	India	61	R	PA	OL	SC	2	OMT
Musa, 2012	[23]	Africa	972	R	PA	OL	MC	3	OCT
Sundar, 2014	[24]	India	500	R	PA	OL	MC	2	OMT
Wasunna, 2016	[25]	Kenya	151	R	PA	OL	SC	3	OCT
Rahman, 2017	[26]	Bangladesh	601	R	PA	OL	SC	4	OCT
Romero, 2017	[27]	Brazil	378	R	PA	OL	MC	3	OCT
Borges, 2017	[28]	Brazil	101	R	PA	OL	SC	2	OMT
Goswami, 2020	[29]	India	154	R	PA	OL	SC	2	OCT

R: randomized, PA: parallel allocation, DB: double blinded, OL: open label, SC: single centric, MC: multicentre, OCT: other combination therapy, OMT: other monotherapy.

2.2. Study selection

For this systematic review and network meta-analysis, only RCTs investigating the efficacy and safety of the antileishmanial interventions were considered. The RCTs were included when VL patients of either gender were enrolled, with following study characteristics: either open-label or blinded, placebo or active comparator, parallel group or crossover, fixed dose or dose ranging, and single- or multi- arm. The different antileishmanial treatment regimens (single drug or combination) included: amphotericin B (AmB), miltefosine, liposomal amphotericin B (L-AmB), paromomycin (PM), meglumine antimoniate (MA), sodium stibogluconate (SSG), sitamaquine, pentavalent antimonials (PA), ketoconazole, and fluconazole. In multi-arm studies of the same drug with different doses, the most appropriate effective dose was included in the analysis to avoid any possible misinterpretation.

The different combination regimens were included as separate treatment nodes in NMA. A placebo or an active treatment was used as comparator. The studies reporting at least one of the following outcomes essentially cure rate, among others as: overall withdrawal rate, relapse rate, frequency of treatment emergent adverse events (TEAEs) and serious TEAEs were included. The RCTs without comparators (comparing different doses of same drug), review articles, case-control studies, cohort studies, case reports, letters, comments, conference abstracts or posters were excluded due to lack of detailed information.

2.3. Data extraction and quality assessment

Two review authors (AB & GS) independently screened the titles and abstracts of initial search results from the electronic databases after excluding the duplicate and irrelevant studies using Endnote software package. After screening for the eligibility, the co-authors retrieved full-text of all potentially relevant articles and extracted maximum possible data. A standardized form in Microsoft Excel (Microsoft, Redmond, WA) was used for data extraction and recording of key outcomes. Two more authors (IR & PT) independently assisted in resolving any discrepancies or inconsistencies between the two review authors. Data collected were; author, publication year, country, study setting, participants' characteristics, details of intervention and reported outcomes.

2.4. Handling of missing data

A few eligible studies did not report all the relevant information like mean±standard deviation or other important variability measures. In such cases, we tried to obtain the information through algebraic back calculation of the available information using the standard formula, or by contacting the authors. Lastly, RCTs with missing data that could not be estimated were excluded in the final analysis.

2.5. Quality assessment

To assess the risk of bias in the included RCTs, we used the 11-item PEDro scale to determine the quality[11]. Two authors (AB and GS) independently assessed the quality of the methodology for each RCT using each item (excluding the item for external validity) and gave a score of (1) or (0) for either present or absent respectively, out of total score of 10. RCTs with score <7 were considered to be at high risk of bias and scoring ⩾7 at low risk of bias.

2.6. Statistical analysis

The main efficacy outcome in this study was success of treatment by antileishmanial interventions as determined by cure rate considering intention-to-treat analysis. The data were synthesized by using the frequentist approach in two stages: pair-wise meta-analyses for direct comparison and NMA for indirect analysis. The effect estimates for included outcomes were reported as *OR* with 95% *CI*s using the random effect model to handle the heterogeneity. Network graphs were formulated to show relationships among different interventions compared for a specific outcome by using "netmeta package" of R programming language version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria)[12]. In the plots, node size represented the sample size and the line thickness between the nodes specified the number of studies included in the comparison. League tables (staircase diagrams) were used to represent all possible comparisons between treatments in the network. The ranking of interventions for the efficacy and safety outcomes was based on p-scores obtained using the surface under the cumulative ranking curve. These scores measure the magnitude of certainty that one treatment is better than the other and the averaged overall competing treatments, while taking the precision into account. Further, statistical heterogeneity across the studies was calculated by using Cochran's *Chi*-squared test (Cochran's Q).

3. Results

3.1. Study selection

Preliminary systematic search from all included databases identified 2 624 potentially relevant trials. After removing the

Table 2. Baseline clinical parameters of the included randomized clinical trials.

No.	Authors, year	Ref.	Intervention	Randomized, *n*	Duration, days	Age, years, mean±SD	Males, *n* (%)	Hb, g/dL, mean±SD	Cr, mg/dL, mean±SD	Fever, *n* (%)	Liver size, cm, mean±SD	Spleen size, cm, mean±SD
1	Seaman, 1993	[13]	SSG (20 mg/kg/d)	67	30	15.2±11.3	39.0 (58.2)	8.2±1.8	NR	NR	1.35±1.9	8.0±3.8
2	Seaman, 1993	[13]	SSG (20 mg/kg/d)+ Aminosidine (PM) (15 mg/kg/d)	67	17	13.8±11.1	29.0 (43.2)	7.6±1.9	NR	NR	1.38±1.90	8.1±5.0
3	Jha, 1998	[14]	Aminosidine (PM) (20 mg/kg/d)	30	21	29.1±14.6	23.0 (76.6)	8.20±1.03	0.1±0.2	30.0 (100.0)	NR	6.3±4.7
4	Jha, 1998	[14]	SSG (20 mg/kg/d)	30	30	26.0±12.5	24.0 (80.0)	8.9±1.3	0.1±0.2	30.0 (100.0)	NR	5.5±3.4
5	Thakur, 2000	[15]	PM (18 mg/kg/d)+SSG (20 mg/kg/d)	48	21	29.1±14.7	31.0 (64.5)	7.3±1.9	NR	48.0 (100.0)	NR	6.3±0.7
6	Thakur, 2000	[15]	SSG (20 mg/kg/d)	50	30	27.9±15.3	42.0 (84.0)	7.4±1.7	NR	50.0 (100.0)	NR	6.2±3.9
7	Sundar, 2002	[16]	Miltefosin (2.5 mg/kg/d)	299	28	26.0±13.0	211.0 (71.0)	8.0±1.5	0.9±0.2	NR	NR	NR
8	Sundar, 2002	[16]	AmB (1 mg/kg/d)	99	29	26.0±12.0	58.0 (59.0)	8.4±1.7	0. 8±0.2	NR	NR	NR
9	Sundar, 2004	[17]	AmB (1 mg/kg/d)	51	30	20.0±2.0	37.0 (73.0)	7.1±0.2	0.9±0.1	51.0 (100.0)	NR	5.0±0.4
10	Sundar, 2004	[17]	L-AmB (2 mg/kg/d)	51	5	17.0±2.0	32.0 (63.0)	8.7±1.3	0.8±0.1	51.0 (100.0)	NR	4.4±0.4
11	Sundar, 2004	[17]	AmB lipid complex (2 mg/kg/d)	51	5	19.0±2.0	37.0 (73.0)	6.9±0.3	0.8±0.1	51.0 (100.0)	NR	4.8±0.5
12	Sundar, 2007	[18]	PM (11 mg/kg/d)	501	21	22.1±12.3	321.0 (64.0)	7.8±1.7	0.8±0.2	99.9 (1.3) ˚F	2.1±1.2	6.6±3.8
13	Sundar, 2007	[18]	AmB (1 mg/kg/d)	165	30	20.8±11.7	95.0 (85.0)	7.7±1.6	0.8±0.2	100.0 (1.4) ˚F	2.1±1.3	6.8±4.0
14	Sundar, 2008	[19]	L-AmB (5 mg/kg)	45	10	27.0±2.0	26.0 (58.0)	7.8±0.3	0.8±0.03	NR	NR	4.2±0.6
15	Sundar, 2008	[19]	L-AmB (5 mg/kg)+Miltefosin (100 mg/kg/d)	45	10	29.0±2.0	29.0 (64.0)	7.5±0.3	0.8±0.02	NR	NR	3.0±0.4
16	Sundar, 2010	[20]	L-AmB (10 mg/kg/d)	304	29	19.0	182.0 (60.0)	8.0±2.0	0.7±0.3	304.0 (100.0)	NR	4.5±3.3
17	Sundar, 2010	[20]	AmB (1 mg/kg/d)	108	29	18.0	67.0 (62)	7.8±1.6	0.7±0.2	108.0 (100.0)	NR	5.2±3.5
18	Hailu, 2010	[21]	SSG (20 mg/kg/d)	135	30	16.7±10.4	101.0 (74.8)	NR	NR	133.0 (98.5)	2.9±2.4	8.2±4.3
19	Hailu, 2010	[21]	PM (15 mg/kg/d)	135	21	17.8±11.1	104.0 (77.0)	NR	NR	133.0 (98.5)	2.9±2.3	8.3±4.9
20	Sundar, 2011	[22]	Sitamaquine (2 mg/kg/d)	41	21	27.5±9.7	23.0 (56.0)	9.6±1.9	0.7±0.1	37.7 (0.9)˚C	NR	15.1±2.2
21	Sundar, 2011	[22]	L-AmB (1 mg/kg/d)	20	30	29.9±9.3	13.0 (65.0)	10.4±2.7	0.7±0.1	37.0 (0.7)˚C	NR	14.4±3.0
22	Musa, 2012	[23]	SSG (20 mg/kg/d)	386	17	15.3±9.3	281 (72.8)	NR	NR	38.1 (1.1)˚C	3.0±2.6	8.1±5.0
23	Musa, 2012	[23]	PM (20 mg/kg/d)	205	21	15.3±9.9	125 (61.0)	NR	NR	38.4 (1.0)˚C	2.8±2.7	7.7±5.0
24	Musa, 2012	[23]	SSG (20 mg/kg/d)+PM (15 mg/kg/d)	381	30	16.1±9.4	273.0 (71.6)	NR	NR	38.2 (1.1)˚C	3.0±2.6	8.0±4.8
25	Sundar, 2014	[24]	AmB (15 mg/kg)	376	30	24.3±14.2	228.0 (60.6)	8.0±2.1	0.83±0.2	NR	NR	6.06±3.92
26	Sundar, 2014	[24]	L-AmB (15 mg/kg)	124	45	26.3±15.2	76.0 (61.3)	4.0±3.2	0.83±0.2	NR	NR	6.3±3.9
27	Wasunna, 2016	[25]	L-AmB (10 mg/kg/d) +SSG (20 mg/kg/d)	51	10	15.0±8.0	37.0 (73.0)	7.4±1.8	0.8±0.2	37.5 (1.1)˚C	2.3±2.4	8.5±4.4
28	Wasunna, 2016	[25]	L-AmB (10 mg/kg/d)+Miltefosin (2.5 mg/kg/d)	49	28	14.0±6.0	14.0 (72.0)	7.0±1.3	0.8±0.2	37.5 (1.1)˚C	2.5±2.3	8.4±5.6
29	Wasunna, 2016	[25]	Miltefosin (2.5 mg/kg/day)	51	28	15.0±8.0	46.0 (90.0)	7.0±1.3	0.8±0.2	37.6 (1.3)˚C	2.7±2.4	8.2±3.9
30	Rahman, 2017	[26]	AmB (5 mg/kg/d)	158	7	22.0±14.5	93.0 (58.9)	8.4±1.4	NR	99.9 (1.7) ˚F	NR	6.2±4.4
31	Rahman, 2017	[26]	AmB (5 mg/kg/d)+PM (2.5 mg/kg/d)	159	10	21.3±14.3	88.0 (55.3)	8.5 ±1.5	NR	99.7 (1.6) ˚F	NR	5.4±3.6
32	Rahman, 2017	[26]	AmB (5 mg/kg/day)+Miltefosin (2.5 mg/kg/day)	142	7	23.5±14.5	98.0 (69.0)	8.6±1.4	NR	99.6 (1.6) ˚F	NR	6.06±3.90
33	Rahman, 2017	[26]	PM (15 mg/kg/d)+Miltefosin (2.5 mg/kg/d)	142	10	19.6±13.4	95.0 (66.9)	8.4±1.3	NR	99.5 (1.6) ˚F	NR	6.2±3.6
34	Romero, 2017	[27]	MA (20 mg/kg/d)	111	20	5.7±6.2	65.0 (58.6)	8.0±1.5	0.5±0.1	111.0 (100.0)	4.0±2.2	6.5±3.7
35	Romero, 2017	[27]	L-AmB (3 mg/kg/day)	109	7	5.6±6.1	59.0 (54.1)	7.9±1.2	0.5±0.1	109.0 (100.0)	4.0±2.2	7.0±3.7
36	Romero, 2017	[27]	L-AmB (10 mg/kg/d) +MA (20 mg/kg/d)	112	20	4.8±5.5	59.0 (52.7)	7.9±1.5	0.5±0.1	112.0 (100.0)	4±2.2	7.0±3.7
37	Borges, 2017	[28]	MA (20 mg/kg/d)	51	20	4.38	25.0 (49.0)	7.8	0.4	48.0 (94.0)	4	6
38	Borges, 2017	[28]	AmB (1 mg/kg/d)	50	14	4.36	31.0 (62.0)	7.6	0.4	44.0 (88.0)	5	8
39	Goswami, 2020	[29]	Miltefosine (2.5 mg/kg/d)	78	28	28.8±13.8	35.0 (44.9)	7.38±1.5	0.8±0.3	NR	3.9±1.4	9.04±3.03
40	Goswami, 2020	[29]	L-AmB (7.5 mg/kg/d)+ Miltefosin (2.5 mg/kg/d)	66	14	32.8±15.2	39.0 (59.1)	7.7±1.2	0.9±0.2	NR	3.5±1.6	8.2±2.5

NR: not reported.

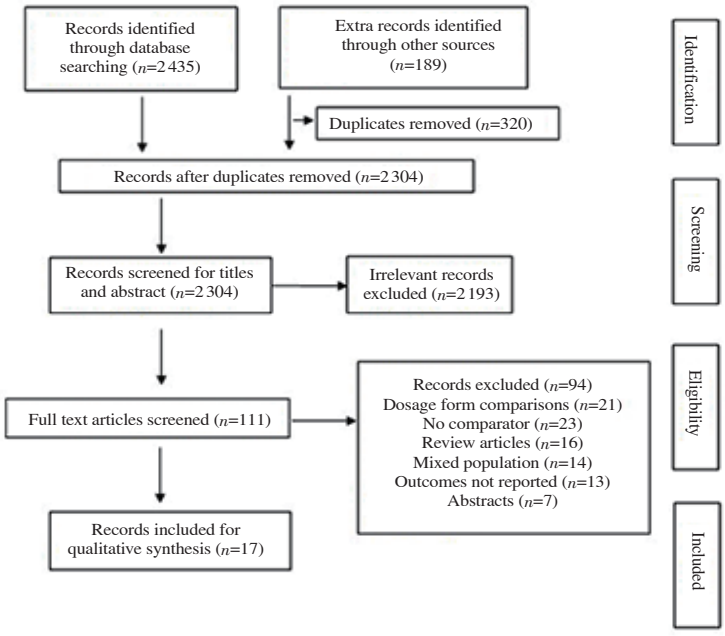

Figure 1. PRISMA flow diagram of study selection process.

duplicate records, a total of 2 304 unique trials were retained and screened for eligibility at stage 1 (title/abstract screening). At stage 2, 2 193 records were excluded and the remaining 111 articles were evaluated for eligibility in full-text version. Among the screened articles, only 17 RCTs met the full inclusion and exclusion criteria[13–29]. Among these 17, 14, 11, 15, and 10 trials assessed cure rate, overall withdrawal, relapse rate, TEAEs and SEAs, respectively. The PRISMA flow diagram of detailed selection process of the included trials in this study is illustrated in Figure 1.

3.2. Study characteristics

Table 1 presents the characteristics of included studies. All the 17 studies were open-label and followed parallel assignment during the conduct of trial. Four studies (23.5%) were multicentric, while 13 (76.5%) trials were single-centre. The included RCTs were conducted in a period of 28 years. Geographically, ten studies were conducted in India, 2 in Brazil, and 1 in each United Kingdom, Sudan, Africa, Kenya and Bangladesh. All the studies had active controls; 9 (52.9%) with active other monotherapy and 8 (47.1%) with other combination therapy. The studies comprised a total of 5 143 VL patients, majority 3 291 (64.0%) were males and the overall mean age was 4.36 to 32.8 years. Each included trial provided two independent datasets for two different comparisons and were considered separately for data analysis. The intervention duration ranged from 5 to 45 days (within L-AmB), and the sample size between the intervention groups ranged from 20 to 501. The detailed characteristics of included RCTs studies are presented in Table 2.

3.3. Quality of the included studies

Based on the PEDro scale quality assessment tool for RCTs, the overall quality of included RCTs was considered with a “Low risk” of bias. The mean score was 7.0, with the key problem items being blinding, and allocation concealment.

3.4. Efficacy outcomes

3.4.1. Cure rate

Cure rate was the primary efficacy outcome in this study and was defined as the percentage of patients with absence of parasites on splenic aspirate after treatment. Seventeen trials involving 15 interventions reported this outcome and were included for analysis (Supplementary Table S3). In the traditional pair-wise meta-analysis (Figure 2), SSG+L-AmB (*OR* 4.16; 95% *CI* 0.28-61.57), AmB+PM (*OR* 3.06; 95% *CI* 0.16-59.86), L-AmB+Miltefosine (*OR* 2.27; 95% *CI* 0.31-16.77), L-AmB (*OR* 1.69; 95% *CI* 0.51-5.55) and L-AmB+MA (*OR* 1.35; 95% *CI* 0.15-11.78) were found to have higher cure rates but did not show a statistical significance as compared to conventional Amphotericin B.

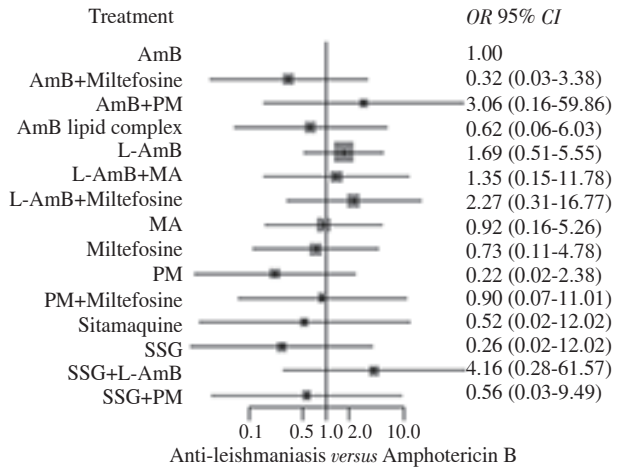

Figure 2. Pair-wise meta-analysis in network meta-analysis for cure rate.

The comparative efficacy of all the 15 interventions is represented in network (Figure 3). No statistically significant differences were observed when compared them indirectly through the network approach. Meanwhile, rankogram analysis (Table 3) revealed that PM (p-score=0.814 8) had the highest probability of being best in the pool, SSG (*OR* 0.82; 95% *CI* 0.24-2.79, p-score=0.758 0) followed by AmB+Miltefosine (*OR* 0.66; 95% *CI* 0.02-19.04, p-score=0.732 9) were superior as compared to the remaining treatments (Table 4).

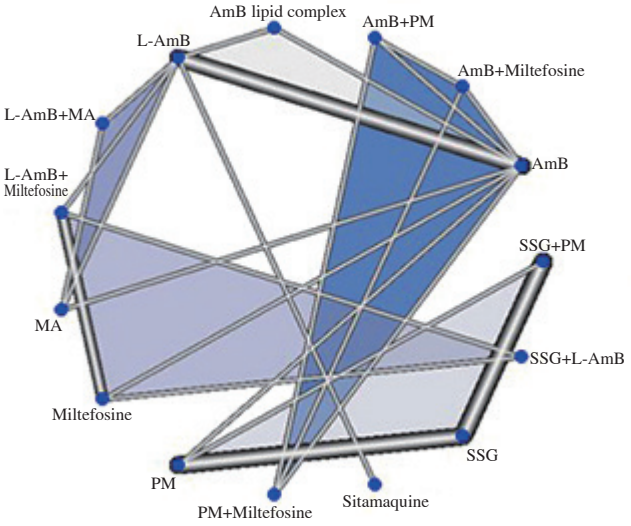

Figure 3. Network plot of interventions reporting cure rate as outcome. AmB: Amphotericin B, L-AmB: liposomal amphotericin B, PM: paromomycin, MA: meglumine antimoniate, SSG: sodium stibogluconate, PA: pentavalent antimonials.

Table 3. Ranking probabilities of interventions reporting cure rate.

Rank	Intervention	p-score
1	PM	0.814 8
2	SSG	0.758 0
3	AmB+Miltefosine	0.732 9
4	Sitamaquine	0.611 5
5	AmB lipid complex	0.587 9
6	Miltefosine	0.570 7
7	SSG+PM	0.564 5
8	MA	0.500 2
9	PM+Miltefosine	0.495 3
10	AmB	0.470 7
11	L-AmB+MA	0.396 5
12	L-AmB	0.307 6
13	L-AmB+Miltefosine	0.264 3
14	AmB+PM	0.247 8
15	SSG+L-AmB	0.177 2

3.4.2. Overall withdrawal

Fourteen trials involving 15 interventions reported overall withdrawal as their outcome (Supplementary Table S4). Among them, pair-wise meta-analysis (Supplementary Figure S1) with reference to AmB showed that AmB+Miltefosine (*OR* 2.68; 95% *CI* 0.32-22.37) had the highest overall withdrawals, followed by MA (*OR* 2.30; 95% *CI* 0.34-15.65), SSG (*OR* 2.00; 95% *CI* 0.10-38.39), PM (*OR* 1.65; 95% *CI* 0.11-24.48), L-AmB+MA (*OR* 1.62; 95% *CI* 0.15-17.29) and SSG+PM (*OR* 1.54; 95% *CI* 0.07-32.57). However, the differences between the included interventions were not statistically significant.

Indirect comparisons of NMA are presented in Supplementary Table S5 and Figure S2, SSG+L-AmB (p-score=0.886 0) showed the highest withdrawals, followed by L-AmB+Miltefosine (*OR* 0.28; 95% *CI* 0.01-7.93, p-score=0.797 7), Sitamaquine (*OR* 0.28; 95% *CI* 0.00-52.92, p-score=0.768 4), and L-AmB (*OR* 0.13; 95% *CI* 0.00-8.10, p-score=0.717 0) as compared to other interventions (Supplementary Table S6).

3.4.3. Relapse rate

Eleven reports with 11 interventions had studied relapse rate as an outcome in the trial (Supplementary Table S7). Traditional pair-wise meta-analysis when compared with AmB showed that SSG+PM (*OR* 51.11; 95% *CI* 0.35-7 431.98) had the highest relapse rate, followed by SSG (*OR* 50.05; 95% *CI* 0.95-2 640.71), PM (*OR* 15.53; 95% *CI* 0.76-317.62), AmB lipid complex (*OR* 7.67; 95% *CI* 0.84-70.36), Miltefosine (*OR* 4.15; 95% *CI* 0.49-38.88), L-AmB (*OR* 2.19; 95% *CI* 0.54-8.93), L-AmB+Miltefosine (*OR* 1.78; 95% *CI* 0.23-14.05) and the remaining interventions are presented in Supplementary Figure S3. None of the compared interventions showed statistically significant differences.

The relative efficacy of all the 11 interventions was shown in

Table 4. League table reporting cure rate (n=17).

Treatments	PM	SSG	AmB+ Miltefosine	Sitamaquine	AmB lipid complex	Miltefosine	SSG+PM	MA	PM+ Miltefosine	AmB	L-AmB+ MA	L-AmB	L-AmB+ Miltefosine	AmB+PM	SSG+ L-AmB
PM															
SSG	0.82 (0.24-2.79)														
AmB+ Miltefosine	0.66 (0.02-19.04)	0.81 (0.02-29.02)													
Sitamaquine	0.42 (0.01-1.74)	0.51 (0.01-32.11)	0.63 (0.01-31.58)												
AmB lipid complex	0.35 (0.01-9.42)	0.42 (0.01-14.40)	0.52 (0.02-13.59)	0.83 (0.02-33.32)											
Miltefosine	0.29 (0.01-6.21)	0.36 (0.01-9.65)	0.44 (0.02-8.92)	0.71 (0.02-23.89)	0.85 (0.05-14.82)										
SSG+PM	0.39 (0.09-1.72)	0.47 (0.14-1.55)	0.58 (0.01-22.86)	0.93 (0.01-63.69)	1.11 (0.03- 41.95)	1.31 (0.04-39.08)									
MA	0.23 (0.01-4.57)	0.29 (0.01-7.16)	0.35 (0.02-6.56)	0.56 (0.02-16.09)	0.68 (0.05-10.21)	0.80 (0.07-9.15)	0.61 (0.02-16.98)								
PM+ Miltefosine	0.24 (0.01-7.74)	0.29 (0.01-11.71)	0.36 (0.03-3.77)	0.58 (0.01-32.22)	0.69 (0.02-20.45)	0.82 (0.04-18.71)	0.62 (0.01-27.39)	1.02 (0.05-21.72)							
AmB	0.22 (0.02-2.38)	0.26 (0.02-3.92)	0.32 (0.03-3.38)	0.52 (0.02-12.02)	0.62 (0.06-6.03)	0.73 (0.11-4.78)	0.56 (0.03-9.49)	0.92 (0.16-5.26)	0.90 (0.07-11.01)						
L-AmB+MA	0.16 (0.01-4.07)	0.20 (0.01-6.25)	0.24 (0.01-5.87)	0.38 (0.01-12.90)	0.46 (0.02-8.88)	0.54 (0.04-8.27)	0.42 (0.01-14.72)	0.68 (0.10-4.77)	0.67 (0.02-18.35)	0.74 (0.08-6.50)					
L-AmB	0.13 (0.01-1.86)	0.16 (0.01-2.99)	0.19 (0.01-2.66)	0.31 (0.02-5.64)	0.37 (0.04-3.57)	0.43 (0.06-3.14)	0.33 (0.02-7.15)	0.54 (0.10-2.86)	0.53 (0.03-8.53)	0.59 (0.18-1.95)	0.80 (0.11-5.70)				
L-AmB+ Miltefosine	0.09 (0.00-2.16)	0.12 (0.00-3.34)	0.14 (0.01-3.11)	0.23 (0.01-7.69)	0.27 (0.02-4.97)	0.32 (0.08-1.37)	0.25 (0.01-7.89)	0.40 (0.03-4.81)	0.39 (0.02-9.76)	0.44 (0.06-3.25)	0.59 (0.04-9.17)	0.74 (0.10-5.36)			
AmB+PM	0.07 (0.00-3.22)	0.09 (0.00-4.79)	0.11 (0.01-1.81)	0.17 (0.00-12.84)	0.20 (0.00-8.59)	0.24 (0.01-8.06)	0.18 (0.00-11.11)	0.30 (0.01-9.44)	0.29 (0.01-5.74)	0.33 (0.02-6.40)	0.44 (0.01-17.47)	0.55 (0.02-13.58)	0.74 (0.02-26.74)		
SSG+L-AmB	0.05 (0.00-1.91)	0.06 (0.00-2.87)	0.08 (0.00-2.77)	0.12 (0.00-6.70)	0.15 (0.00-4.63)	0.18 (0.02-1.47)	0.13 (0.00-6.70)	0.22 (0.01-4.84)	0.22 (0.01-8.55)	0.24 (0.02-3.56)	0.32 (0.01-8.86)	0.41 (0.03-6.17)	0.55 (0.06-4.64)	0.73 (0.01-40.66)	

network (Supplementary Figure S4) and no statistically significant differences were observed in relapse rate among the included interventions when compared indirectly in NMA. Further, rankogram analysis revealed that L-AmB+MA (p-score=0.910 4) is associated with the highest rate of relapse as compared to SSG+L-AmB (*OR* 0.71; 95% *CI* 0.02-29.94, p-score=0.864 4), AmB (*OR* 0.22; 95% *CI* 0.02-3.26, p-score=0.718 2), MA (*OR* 0.22; 95% *CI* 0.02-2.43, p-score=0.700 8) and other compared interventions showed non-significant differences (Supplementary Tables S8 and S9).

3.5. Safety outcomes

3.5.1. Treatment-emergent adverse events (TEAEs)

Fifteen studies reported TEAEs as an outcome for included interventions (Supplementary Table S10). In the conventional pair-wise meta-analysis (Supplementary Figure S5), with reference to AmB, AmB+Miltefosine (*OR* 1.96; 95% *CI* 0.44-8.61), was associated with the highest number of TEAEs, followed by Miltefosine (*OR* 1.81; 95% *CI* 0.51-6.71), PM+Miltefosine (*OR* 1.50; 95% *CI* 0.34-6.61), SSG (*OR* 1.41; 95% *CI* 0.25-8.03), AmB lipid complex (*OR* 1.22; 95% *CI* 0.22-6.80), AmB+PM (*OR* 1.12; 95% *CI* 0.25-4.95). However, the differences between these comparisons were not statistically significant.

Further indirect comparative safety presented through network diagram (Supplementary Figure S6) between the included interventions is revealed through NMA and the rankgram analysis (Supplementary Table S11 and S12). The results show that Sitamaquine (p-score=0.999 5) was associated with highest number of TEAEs followed by L-AmB (*OR* 0.02; 95% *CI* 0.00-0.16, p-score=0.767 0), MA (*OR* 0.02; 95% *CI* 0.00-0.26, p-score=0.708 5), SSG+L-AmB (*OR* 0.02; 95% *CI* 0.00-0.28, p-score=0.657 0) and other interventions reported in the Supplementary Table S11.

3.5.2. Serious treatment-emergent adverse events (SAEs)

Ten studies with 11 interventions reported SAEs as an outcome. Pair-wise meta-analysis shown that SSG+L-AmB (*OR* 3.96; 95% *CI* 0.01-2 633.92) was associated with the highest number of SAEs, followed by L-AmB+Miltefosine (*OR* 2.26; 95% *CI* 0.01-684.82) and Miltefosine (*OR* 2.01; 95% *CI* 0.02-210.13) when compared to AmB as a reference, but the differences were not statistically significant (Supplementary Figure S7).

The indirect safety comparison between these interventions was shown in network (Supplementary Figure S8). The NMA and rankogram results reveal that L-AmB (p-score=0.695 3) showed the highest number of SEAs, followed by AmB lipid complex (*OR* 0.54; 95% *CI* 0.01-27.84, p-score=0.575 6), SSG+PM (*OR* 0.45; 95% *CI* 0.00-432.51, p-score=0.551 1), L-AmB+MA (*OR* 0.44; 95% *CI* 0.01-23.41, p-score=0.543 8) and other compared interventions with non-significant differences between them (Supplementary Table S13 and S14).

3.6. Heterogeneity assessment

A significant heterogeneity was observed for the cure rate in the overall network (Q_{total}=40.60, P<0.001), which could be further crumbled into a non-significant heterogeneity between the designs ($Q_{between}$=10.01, P=0.124) and significant heterogeneity within the designs (Q_{within}=30.59, P<0.001).

4. Discussion

This systematic review and network meta-analysis compared the efficacy and safety outcomes of different interventions recommended in the management of VL using a frequentist approach. We observed that many interventions could be used potentially to have increased proportion of patients with better cure rates; but none of the studies had an adequate sample size to confirm their pooled estimates. In this NMA, a total of 17 publications related to 15 interventions involving 5 143 VL patients were included. As per available literature, this is the first comprehensive and effective analysis performed to date for comparing active treatments used in VL both directly and indirectly. With our main outcome, the NMA results showed that Paromomycin had the top rank with the highest cure rate in VL patients, followed by Sodium stibogluconate, Amphotericin B+Miltefosine and Sitamaquine among the all 15 compared interventions recommended. However, by using pair-wise meta-analysis, combined therapy with SSG+L-AmB was reported to be significantly superior in achieving the best cure rates. Meanwhile in both the approaches of analysis, the differences were statistically non-significant and further considering the large confidence interval for included outcomes, these results need to be deciphered cautiously.

Our NMA results are consistent with the previous reports[14,23,26] and a recent systematic review by Pokharel *et al*[9], reporting the use of PM alone or in combination with other agents to have high cure rates and better tolerability. Further, this study confirms the previous efforts by furnishing better understanding to the current body of evidence on relative effectiveness of recommended interventions for VL. As an off-patent aminoglycoside antibiotic, Paromomycin is internationally available in three dosage forms; oral, topical, and

parenteral used in both bacterial and parasitic infections[30].

Presently available meta-analyses in this topic have evaluated a limited number of interventions. Results of a meta-analysis reported by Rodrigo *et al*., on different formulations of Amphotericin B, concluded that both AmB deoxycholate and ABLC (lipid complex) are as effective as Liposomal-AmB. However, the lipid complex was better than AmB emulsion[31]. Further, they also reported that the efficacy of Paromomycin or Miltefosine (as monotherapy or in combination with L-AmB) was similar to that of AmB deoxycholate alone or L-AmB alone. Additionally, they demonstrated that AmB deoxycholate was superior to antimonial compounds in achieving definitive cure in India and recommended more prospective trials at other geographical locations.

Another meta-analysis by Eyob *et al*. has suggested the use of the combination of SSG with PM over SSG monotherapy[32]. The authors further suggest the use of this combination with multiple doses of L-AMB especially among patients with other complications, any severe disease, HIV co-infection, and intolerance to the adverse effects of antimonials. Meanwhile, our study is the largest meta-analysis involving maximum number of studies and patients in VL with a comparative analysis of all these interventions in a single platform.

Reporting on the relapse of VL cases after being treated with different antileishmanial agents, combined therapy with L-AmB plus MA ranked first while SSG has shown a minimum relapse of cases with its use. Additionally, the comparative analysis showed that SSG+L-AmB ranked first and L-AmB+Miltefosine second, and Sitamaquine third for overall withdrawals from the clinical trials. In our study, most of the TEAEs were reported with Sitamaquine and least TEAEs were reported with the AmB+Miltefosine. Furthermore, SEAs were found to be at a maximum with L-AmB and least with SSG+L-AmB. In contrast to our findings, one recent meta-analysis by Sauman *et al*. analyzed SEAs with different interventions used in VL management and reported that maximum (39.2%) of SEAs were seen among patients treated with PA, 16.5% among those treated with miltefosine and 10.1% among those treated with AmB from all other antileishmanial[33]. The most commonly found SEAs associated with antileishmanial agents are reported as cardiac disorders, infections and infestations, blood and lymphatic disorders, and gastrointestinal disorders[34]. To summarize, all the VL patients need safe, effective, affordable, and accessible treatment, the investigators involved in the VL management shall consider comparing the therapeutic outcomes with various interventions as per ranks observed in the present meta-analysis. This network meta-analysis, like any other method, has its own limitations. Limited number of trials within each pair-wise direct comparison did not allow to evaluate statistical heterogeneity/inconsistency. Secondly, variation in geographical location and inherent variations in treatment duration, dosage, dosage forms, large confidence intervals, *etc*. might have added some bias to results of this indirect comparison. Thirdly, several RCTs in which the data of included outcomes was reported lacked a comparison group; and, hence were excluded as it is a basic requirement to include a study in NMA. Finally, due to limited number of trials in each intervention, our network meta-analysis led to statistically insignificant difference between treatments compared. However, rankogram analysis helped to rank the best intervention among the pool for each outcome.

5. Conclusions

The present study has evaluated the multiple available treatment options recommended in visceral leishmaniasis management and provided the effect size estimates despite the absence of head-to-head clinical studies. Paromomycin reported the advantage in comparison to other agents in achieving higher cure rates. L-AmB plus MA combination was associated with high relapse rates while L-AmB alone reported the maximum SEAs. Future research with direct head-to-head RCTs and timely update of new findings is warranted to further strengthen these results.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

AB and GS contributed in literature search and selection process. AB, GS, IR and PT contributed towards the conception of the article, data analysis, quality assessment, drafting and reviewed the manuscript, performed critical revisions related to important intellectual content of the manuscript. PT contributed to the final revision of the manuscript. All authors read and approved the final manuscript.

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