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Effect of Resveratrol and Its Derivatives on *Leishmania* Viability: A Meta-Analysis

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ABSTRACT

Background & Objective: Leishmaniasis is among the seven more significant tropical diseases, and it is a major global health issue with a wide range of clinical symptoms and potentially lethal consequences. Resveratrol and its derivatives have been shown to have anti-Leishmanial properties. This study aimed to use a meta-analysis of relevant papers to determine the leishmanicidal impact of resveratrol and its derivatives.

Materials & Methods: A comprehensive search method was used to query the electronic databases of PubMed, ScienceDirect, Embase, ISI Web of Science, and Scopus up until June 2021. The articles that met the inclusion criteria were chosen. Random-effects models were used to calculate mean differences in IC50 (concentration corresponding to a 50% reduction in *Leishmania*) for each outcome. The Newcastle-Ottawa Scale was used to assess the quality of the evidence. To assess heterogeneity and the stability of the pooled data, sensitivity and subgroup analyses were performed. The Egger's and Begg's tests were used to assess publication bias.

Results: In the meta-analysis, nine studies were considered. Resveratrol (RSV) and its derivatives significantly reduced survivability in *Leishmania* promastigote [24.02 µg/ml; (95% CI 17.1, 30.8); $P<0.05$; $I^2 = 99.8\%$; $P_{\text{Heterogeneity}} = 0.00$] and amastigote [18.3 µg/ml; (95% CI 13.5, 23.2); $P<0.05$; $I^2 = 99.6\%$; $P_{\text{Heterogeneity}} = 0.00$]. The meta-analysis revealed a considerable publication bias. Sensitivity analyses revealed that the effect magnitude was similar, but the heterogeneity was reduced. According to subgroup analysis, the pooled effect sizes of leishmanicidal resveratrol and its derivatives were altered by the kind of stilbenes and *Leishmania* species.

Conclusion: According to the findings of this meta-analysis, RSV and its derivatives could be a possible therapeutic option for leishmaniasis. However, more research is needed to confirm and employ this chemical against *Leishmania*.

Keywords: Resveratrol, Stilbenes derivatives, *Leishmania*, Leishmaniasis, Meta-analysis

Introduction

Leishmaniasis is a worldwide parasitic disease that involves various diseases such as cutaneous, mucocutaneous, and visceral manifestations. In 2020, 98 nations out of 200 reporting to the WHO were endemic for leishmaniasis. This covers 71 nations with both VL and CL endemics, eight with solely VL endemics, and 19 with only CL endemics (1). Over 350 million people are at risk worldwide, with 0.7–1.3 million new cases reported annually (2). CL caused by *L. major*, *L. amazonensis*, *L. panamensis* and *L. tropica* species and *L. donovani,* and *L. infantum* are associated with visceral leishmaniasis (VL) (3). *Leishmania* parasite has a digenetic life cycle, including the extracellular promastigote in sand-flies and the intracellular amastigote in the mammalian hosts. Although extensive research has been conducted to develop new therapeutic targets and vaccine candidates for leishmaniasis, no vaccine is currently available for the illness (4, 5). Leishmaniasis is now treated with chemotherapy using pentavalent antimonials such as sodium stibogluconate (pentostam) and meglumine antimoniate (glucantime) (6). In India, paromomycin and amphotericin B were reintroduced to treat refractory VL when previous medicines failed (7). Liposomal amphotericin B, amphotericin B lipid

complex, and amphotericin B cholesterol dispersion are the three formulations that have been widely investigated in VL (8). The exact mechanism of action of these medicines is unknown. However, other investigations have shown that these medicines reduce ATP synthesis by inhibiting fatty acid glycolytic and oxidative activities (9). Alternative therapy is necessary due to the high toxicity, high cost, and adverse effects of conventional medications and the emergence of drug-resistant strains (10). Miltefosine has been used for visceral leishmaniasis in India since 2002, but its efficacy against visceral leishmaniasis is limited; hence it was replaced with liposomal amphotericin B for kala-Azar therapy in India approximately a decade ago (11). Due to the limitations of chemotherapy and the absence of a viable leishmaniasis vaccine, new medications with greater efficacy and fewer significant side effects are needed (12). Recently, herbal medications have received much attention worldwide, and their medicinal potential has been proven in variousin vitro and in vivo settings (13). Resveratrol (3, 5, 4-trihydroxy-trans-stilbene) has been shown to have antibacterial, antiviral, and antiparasitic properties (14). Resveratrol is a polyphenol chemical compound generated by various plant species such as pines, berries, and peanuts and is primarily found in the skin of grapes and red wine. Its health benefits have been examined for various ailments (15). In nature, RSV represents Cis and Trans isomeric forms, with Tran's form being related to the biological activity (16). Despite several studies about the potential activity of resveratrol against both promastigotes and amastigotes of *Leishmania* parasites (17), research on its anti-leishmanial activity is still in its infancy. Furthermore, resveratrol or its analogs may be a novel potential medicine in the treatment of leishmaniasis in the future. The construction of meta-analysis research, which statistically mixes and examines the data of numerous studies to produce a more trustworthy output, is one technique to get a conclusive result (18). Despite prior studies linking resveratrol and other stilbene compounds to leishmanicidal actions, no systematic evaluation of available data has been conducted. In this work, we conducted the first systematic review and meta-analysis of the leishmanicidal activity of resveratrol and its derivatives. The meta-analysis was conducted in accordance with the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (19).

Materials and Methods

Search Protocol

We performed an exclusive search from October 2000 to June 2020 through Medline, Embase, Scopus, Science Direct, Web of Science, EMBASE, and Cochrane Library. All eligible studies related to the leishmanicidal effects of resveratrol and its derivatives were selected. Also, gray literature and reference lists were reviewed to identify relevant studies. We searched databases using Mesh terms and keywords ("*Leishmania*" OR "leishmaniasis" OR "*Leishmania* species") OR ("*L. major*" OR "*L. tropica*" OR "*L. infantum*" OR "*L. amazonesis*" OR "*L. braziliensis*" OR "*L. donovani*" OR "*L. aethiopica*") AND ("Resveratrol" OR "resveratrol derivatives" OR "resveratrol analogs" OR "trans-resveratrol" OR "3, 5, 4'-trihydroxystilbene"). Furthermore, manual searches of grey literature, conference abstracts, and reference lists were conducted to find possibly relevant research. The language of the searches was confined to English. Two reviewers reviewed each publication individually, and any discrepancies in extracted data were handled by agreement and discussion with a third reviewer. Ethical approval and informed consent will not be applied for because the relevant data we extracted does not involve any individual privacy.

Selection Criteria of Studies

Articles were carefully selected if they met the following criteria: report outcome (the effect of resveratrol and resveratrol analogues on promastigote and amastigote stages of *Leishmania*, English language, published studies in a number of internationally indexed journals and having sufficient information and repetitive articles, studies without limitations in time and the form of resveratrol usage and also studies that reported the IC50 (The compound concentration causing 50% reduction in parasite viability) of resveratrol and its analogues and control's IC50. The absence of relevant study technique details was ruled out.

Quality Assessment

A systematic bias assessment in the included studies was performed independently by two expert authors using Newcastle-Ottawa Scale (NOS) quality assessment (20). In cases of disagreement, the papers would be examined by a third author. Authors discussed the results comprehensively until they agreed on the accuracy and usefulness of data. The items used for the assessment of each study were composed of three main sections coupled with questions within each part: 1) selection, 2) comparability and 3) exposure. NOS score $\geq$ 5 were considered high quality.

Extracted Data of Studies

Using a specifically constructed data extraction form, we extracted data from the final included articles. The form was piloted and tested in three papers, with all three writers extracting data from it. The form was changed as a result of the pilot study. The first writer, the year of publication, the source region, the parasite species, the type of stilbenes (resveratrol and its derivatives), the dose of resveratrol and its derivatives (mg/mL), the IC50 value of resveratrol, the IC50 values of resveratrol derivatives, and the time of exposure, the control's name, the control's IC50, and the quality assessment score were all on the data extraction. Table 1 summarises the extracted data for the promastigote and amastigote phases of *Leishmania*.

Statistical Analysis

The mean and 95% confidence intervals of the half-maximal inhibitory concentration (IC_{50}) values were evaluated by pooling results from all selected articles.

Regarding the existing heterogeneity among studies, this meta-analysis was performed using the random-effects model. In this meta-analysis, both the Q-Cochran test ($p < 0.1$ indicate heterogeneity) and the I^2 method ($I^2 < 50\%$ no heterogeneity and $I^2 > 50\%$ indicate heterogeneity) were used todetect heterogeneity. We also applied the Begg's rank correlation test and the Egger's regression asymmetry test to evaluate the potential publication bias obtained by the funnel plot (21). The trim-and-fill analysis was used to adjust any significant publication bias detected. To establish the possible sources of heterogeneity between the studies on meta-analysis outcome, subgroup analyses were performed based on *Leishmania* species .In addition, conducted sensitivity analyses to evaluate the influence of individual articles on the pooled results. Finally, meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ).

Results

The results of literature search

The search identified 97 records. These included 8 duplicate articles, 5 reviews and 1 editorial, which were removed, leaving 83 unique articles to be screened by title and abstract. Out of the 83 articles screened, 53 were excluded as they did not meet the eligibility criteria: 22 articles were performed on other parasites, 31 articles were not measured the leishmanicidal impact of resveratrol and its derivatives. The full text of 30 articles were then evaluated out of which 21 articles were excluded for the following reasons: i) incomplete reported data, and ii) brief report and review article. Finally, 9 studies met the inclusion criteria for meta-analysis (Figure 1).

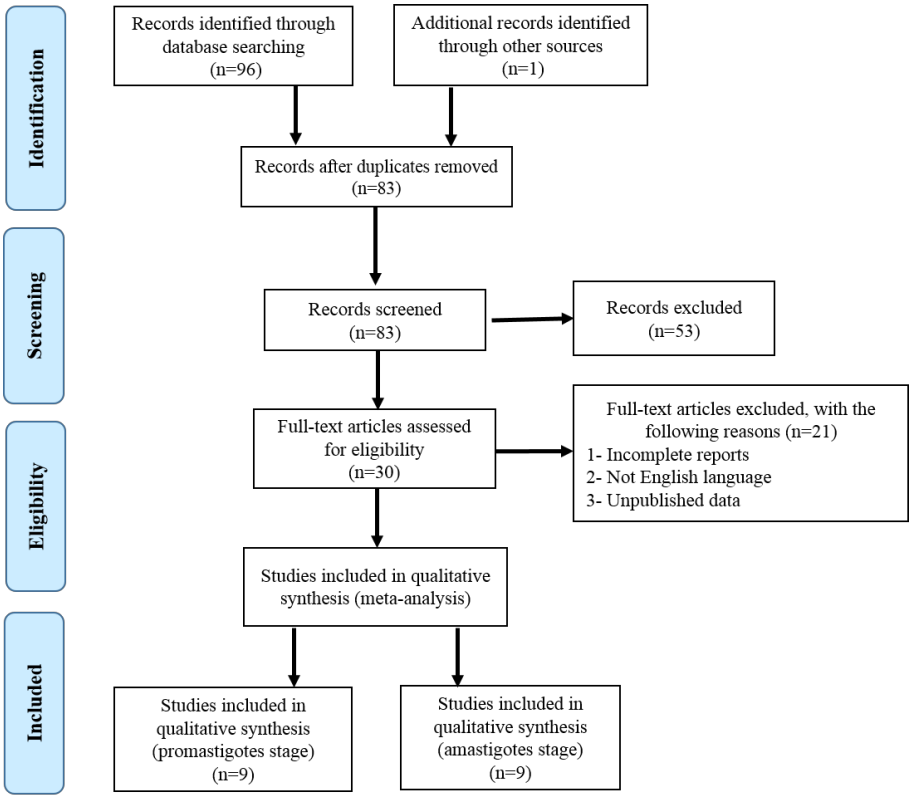


Figure 1. Flow chart of the number of studies detected and selected into the meta-analysis.

Main Characteristics of Eligible Studies

We found nine publications that reported the IC50 of RSV and other stilbenes, and their derivatives, for leishmanicidal activity. Table 1 lists the included papers and their features in this meta-analysis on the promastigote and amastigote stages of *Leishmania*, Most of the research on promastigotes has been done on L. major and L. amazonesis, respectively. Coimbra et al. investigated three species (L. amazonesis, L. braziliensis, and L. major). In contrast, Bruno et al. investigated five species (L. major, L. tropica, L. Donovani, L. Amazonesis, and L. Aethiopica). Brazil (n = 4), Italy (n = 2), Poland (n = 1), Germany (n = 1), and Japan (n = 1) were the sites where the promastigotes experiments were done. In the case of promastigotes, five studies reported both resveratrol and its analogues, with two studies reporting only RSV and three studies reporting only RSV analogs. It should be emphasised that part of the research included data from both RSV and analogs. Other stilbenes were included in the remaining experiments. L. major (n = 3), L. infantum (n = 2), and L. amazonesis (n = 4) have all been studied as amastigotes. The nations that performed the investigations were Poland (n = 1),

Germany (n = 1), Italy (n = 3) and Brazil (n = 4). Three studies reported solely RSV, and six studies reported RSV analogues and other stilbene derivative findings in the field of amastigotes. Amphotericin B, pentostam, and miltefosine were utilised as positive controls for *Leishmania* in the majority of the studies included in this meta-analysis. Table 1 summarizes the quality rating of papers included in our meta-analysis. Each of the three sections of the NOS criteria revealed a low to high risk in all included studies. The quality of the research included in this review ranged from low to high.

Table 1. Baseline Characteristics of included studies the leishmanicidal effects of resveratrol and its derivatives on *Leishmania* in the systematic review and meta-analysis.

Parasite species	Type of stilbenes	Dose (mg/ml)	Exposure IC50 (mg/ml)	Exposure time(h)	Control IC50 (mg/ml)	Country	Quality assessment	Ref
L. major	RSV	10, 50, 100	45	48	-	Poland	4	(22)
L. major	Hydroxylated analogs of RSV	5 10	40.1	48	-		4	
L. major	RSV	45	196.9	48	1	Germany	5	(17)
L. major	*Lonchocarpus nicou* stilbenes (compound1)	50 (mL)	5.5	48	4	Japan	6	(14)
L. major	*Lonchocarpus nicou* stilbenes (compound4)	50 (mL)	3.9	48	4		6	
L. amazonesis	RSV	100	27	48	0.108	Brazil	5	(23)
L. amazonesis	Pterostilbene	-	18	48	0.1	Brazil	6	(24)
L. amazonesis	Piceatannol	-	65	48	0.1		6	
L. amazonesis	Polydatin	-	95.5	48	0.1		6	
L. amazonesis	oxyresveratrol	-	65	48	0.1		6	
L. amazonesis	RSV analoges	1.56-25	3.81	24	8.56	Brazil	7	(25)
L. braziliensis	RSV analoges	1.56-25	1.60	24	11.44		7	
L. major	RSV analoges	1.56-25	7.84	24	8.15		7	
L. infantum	trans stilbenes derivatives	4	2.1	48	2.1	Italy	6	(26)
L. major	trans stilbenes (ST18)	15	14.2	48	14.9	Italy	5	(27)
L. aethiopica	trans stilbenes (ST18)	3	2.9	48	2.9		5	
L. donovani	trans stilbenes (ST18)	3	3.2	48	>50		5	

Parasite species	Type of stilbenes	Dose (mg/ml)	Exposure IC50 (mg/ml)	Exposure time(h)	Control IC50 (mg/ml)	Country	Quality assessment	Ref
L. tropica	trans stilbenes (ST18)	3	2.5	48	19.5		5	
L. amazonesis	trans stilbenes (ST18)	15	14.4	48	15.4		5	
L. braziliensis	trans stilbenes (ST18)	6	4.3	48	8.2		5	
L. amazonesis	RSV analoges (AR27)	0.4-30	2.6	72	0.11	Brazil	6	(28)
L. braziliensis	RSV analoges (AR27)	0.4-30	0.7	72	0.12		6	
L. infantum	RSV analoges (AR26)	0.4-30	3	72	0.05		6	
L. major	RSV	40 45	20	48	-	Poland	4	(22)
L. major	RSV	9.9	43.6	48	1	Germany	5	(17)
L. infantum	TTAS	-	4.3	48	7	Italy	4	(29)
L. amazonesis	RSV	200	42	24	0.0088	Brazil	5	(23)
L. amazonesis	Pterostilbene	-	33.2	24	8.8	Brazil	6	(24)
L. amazonesis	Piceatannol	-	45	24	8.8		6	
L. amazonesis	Polydatin	-	29	24	8.8		6	
L. amazonesis	oxyresveratrol	-	30.5	24	8.8		6	
L. amazonesis	RSV analoges	1.56-25	5.73	24	2.2	Brazil	7	(25)
L. infantum	trans stilbenes derivatives	1	0.81	48	2.1	Brazil	6	(26)
L. major	trans stilbenes (ST18)	1.0 - 24	16.3	48	10.4	Italy	5	(27)
L. aethiopica	trans stilbenes (ST18)	1.0 - 24	3	48	3.2		5	
L. donovani	trans stilbenes (ST18)	1.0 - 24	5.8	48	24.8		5	
L. tropica	trans stilbenes (ST18)	1.0 - 24	12.5	48	16.8		5	
L. amazonesis	trans stilbenes (ST18)	1.0 - 24	13.4	48	12.8		5	
L. braziliensis	trans stilbenes (ST18)	1.0 - 24	2.6	48	4.2		5	
L. braziliensis	RSV analoges (AR26)	3.1-50	15.9	72	0.069	Italy	6	(28)

[a]RSV: Resveratrol, [b]Ref: References.

Quantitative Data Synthesis

Leishmanicidal Effects of Resveratrol and Its Derivatives

Figure 2 presents pooled mean findings of IC50 for leishmanicidal effects of resveratrol and its derivatives in both the promastigote and amastigote stages of

Leishmania. Based on random-effects model, the pooled mean of IC_{50} were observed for promastigote stage [24.02 µg/ml (95% CI 17.1, 30.8) $P < 0.05$] and amastigote stage [18.3 µg/ml (95% CI 13.5, 23.2) $P < 0.05$] following treatment of resveratrol and resveratrol derivatives. These results showed that resveratrol and its derivatives significantly reduced *Leishmania* viability in both promastigote and amastigote stages of *Leishmania*. There was a statistically significant heterogeneity between studies [($I^2 = 99.8\%$, $P < 0.05$)].

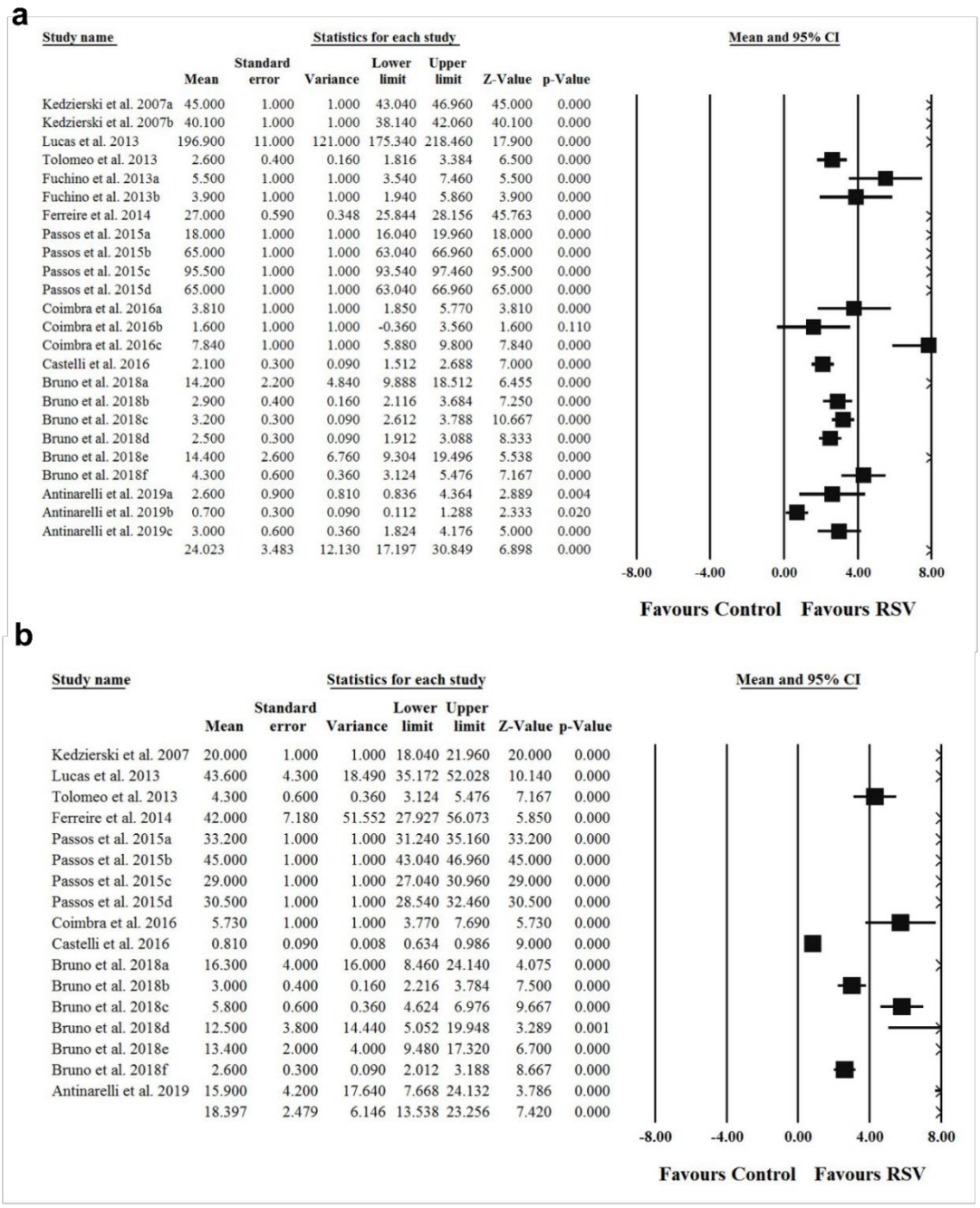


Figure 2. Forest plot evaluating mean of IC50 and 95% confidence intervals for the leishmanicidal effects of RSV and other stilbenes derivatives in a) promastigote and b) amastigote stages of *Leishmania*. Meta-analysis was performed using a random-effects model with inverse variance weighting.

Publication Bias

The Begg's rank correlation tests (Kendall's Tau with continuity correction = 0.52, Z= 3.5, two-tailed *P*-value< 0.001) and the Egger's linear regression tests (intercept = 29.9, standard error = 9.4; 95% CI = 10.2, 49.5, t = 3.1, df = 22, two-tailed $P = 0.004$) were statistically significant. Thus, the funnel plot of the study precision (inverse standard error) by effect size (mean IC50) was asymmetric and indicated potential publication bias in reporting the leishmanicidal effects of resveratrol and its

derivatives on promastigote and amastigote stages of *Leishmania*. The observed publication bias was imputed using trim-and-fill correction. Trim-and-fill correction imputed 15 potentially missing studies resulted in a corrected effect size of (5.5; 95% CI: -2.8, 13.8) and (2.7; 95% CI: -2.3, 7.8) for promastigote and amastigote stages of *Leishmania,* respectively. According to "fail safe N" method, 60969 and 14887 theoretically missing studies were required to bring p-value to> 0.05 in both promastigote and amastigote stages, respectively (Figure 3).

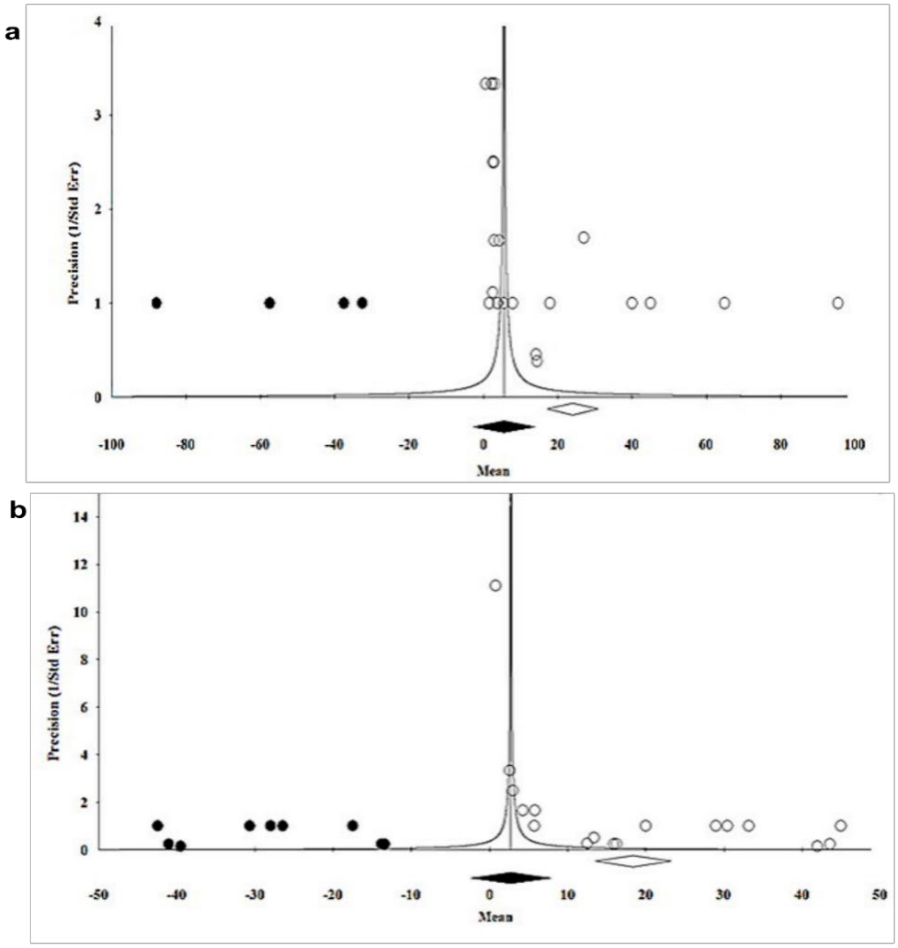
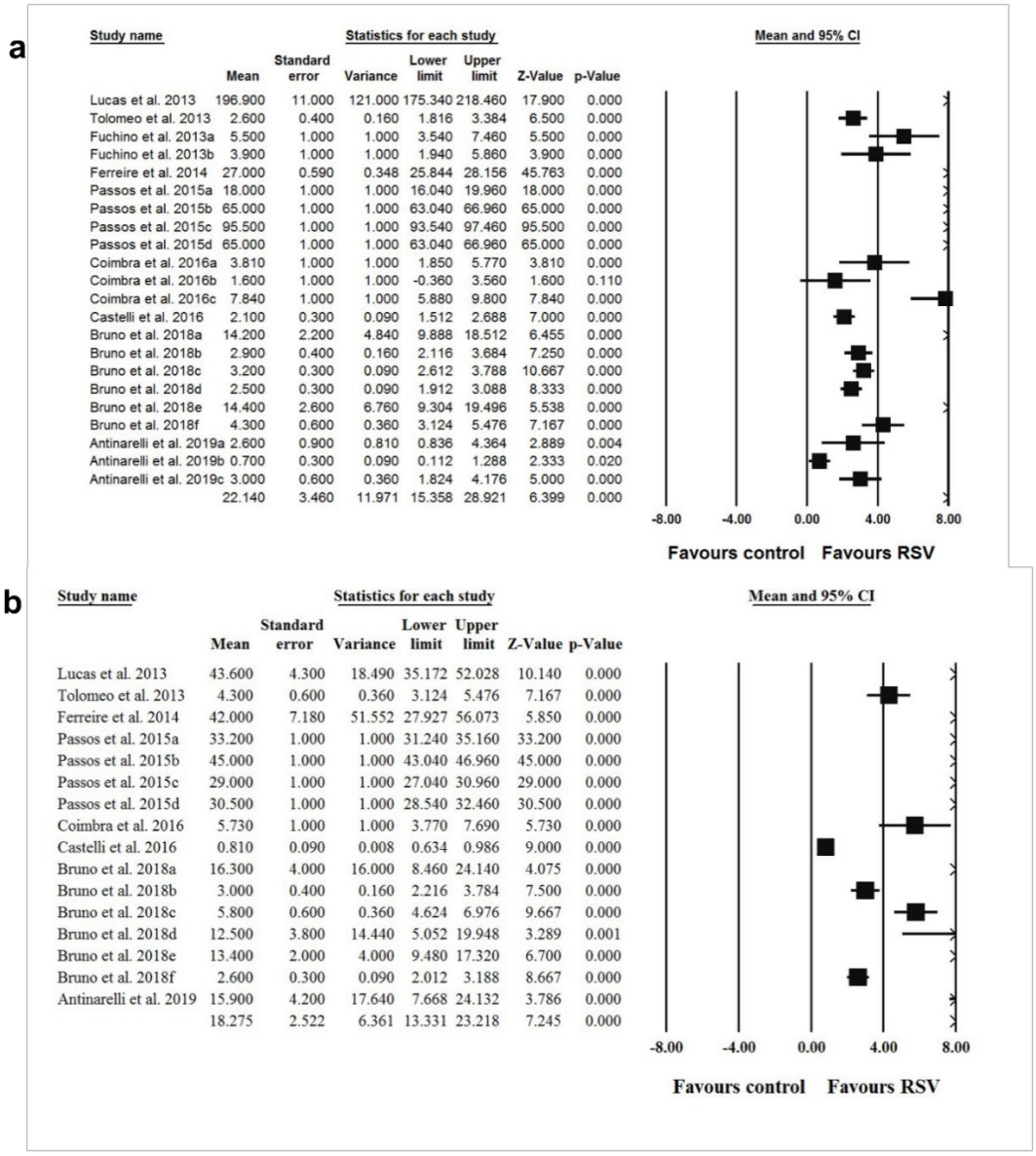


Figure 3. Random-effects Funnel plot detailing publication bias in the studies investigating the leishmanicidal effects of RSV and other stilbenes derivatives in a) promastigote and b) amastigote stages of *Leishmania* after trimming and filling. Circles represent observed published studies; closed circles represent imputed unpublished studies.

Sensitivity Analysis

We ran a sensitivity analysis when removing any research from our meta-analysis. In the sensitivity analysis, it was discovered that removing one research from the analysis using the "leave-one-out method" had no significant effect on the outcome (mean of IC50) compared to the total effect size for the leishmanicidal effects of resveratrol and other stilbene derivatives in promastigote [22.1 µg/ml (95% CI 15.3, 28.9) $P < 0.05$] and amastigote [18.2 µg/ml (95% CI 13.3, 23.2) $P < 0.05$] (supplementary Figure 1). Therefore, this pooled analysis outcome could be regarded as a higher degree of certainty.



Supplementary Figure 1. Leave-one-out sensitivity analysis of the leishmanicidal effects of RSV and other stilbenes derivatives in a) promastigote and b) amastigote stages of *Leishmania*. Meta-analysis was performed using a random-effects model with inverse variance weighting.

Subgroup Analysis

Subgroup analysis was performed according to *Leishmania* species (*L. major*, *L. amazonesis*, *L. braziliensis*, *L. donovani* and *L. infantum*) and type of stilbenes (resveratrol and its derivatives and stilbenes derivative). The subgroup analysis revealed significantly higher levels of leishmanicidal effects of resveratrol and its derivatives in *L. major* [40.08 µg/ml; 95% CI (24.1, 56); $P < 0.05$)], *L. amazonesis* [36.4 µg/ml; 95% CI (13.6, 59.1); p= 0.002)] and *L. infantum* [2.3 µg/ml; 95% CI (1.9, 2.8); $P< 0.05$)] of promastigote stage, while, it had no significant leishmanicidal effect in *L. braziliensis* [2.1 µg/ml; 95% CI (-0.31, 4.7); $P = 0.08$)]. Furthermore, in amastigote stage of *Leishmania*, the leishmanicidal effects of resveratrol and its derivatives were significantly revealed in L. major [26.2 µg/ml; 95% CI (12.8, 39.7); $P < 0.05$)] and *L. amazonesis* [28.03 µg/ml; 95% CI (17.3, 38.7); $P < 0.05$)]; although, no significant increases in *L. infantum* [2.5 µg/ml; 95% CI (-0.91, 5.9); $P = 0.15$)] and *L. braziliensis* [8.5 µg/ml; 95% CI (-4.3, 21.5); $P = 0.19$)] was observed. On the other hand, the results of subgroup analyses showed that the leishmanicidal effects were significantly increased in subgroups of RSV and its derivatives and stilbene derivatives in both promastigote and amastigote stages of *Leishmania* (Table 2). Taken together, subgroup of resveratrol and its derivatives in both stages of *Leishmania* significantly reduced *Leishmania* viability.

Table 2. Evaluation of the leishmanicidal effects of resveratrol and its derivatives on promastigote and amastigote stages of *Leishmania* using subgroup analysis.

Subgroup	Number of comparisons	Mean (µg/ml) (95% CI)	Z-value	*P*	Test of Heterogeneity	
					I^2 (%)	*P*
Promastigote						
Overall	9	24.02 (17.1, 30.8)	3.1	<0.05	99.8	<0.05
***Leishmania* species**						
L. major	7	39.7 (24.04, 55.5)	4.9	<0.05	99.7	<0.05
L. amazonesis	6	32.8 (11.61, 54.01)	3.03	0.002	99.9	0.05
L. infantum	2	2.4 (1.5, 3.2)	5.6	<0.05	44.4	<0.05
L. braziliensis	3	2.1 (-0.31, 4.7)	1.7	0.08	93.06	<0.05
Type of stilbenes						
RSV & its derivatives	6	39.3 (24.6, 54.1)	5.2	<0.05	99.9	<0.05
Stilbenes derivatives	10	3.8 (2.9, 4.7)	8.5	<0.05	87.06	<0.05
Amastigote						
Overall	9	18.3 (13.5, 23.2)	4.5	<0.05	99.6	<0.05
***Leishmania* species**						
L. major	3	26.2 (12.8, 39.7)	3.8	<0.05	93.3	<0.05
L. amazonesis	7	28.03 (17.3, 38.7)	5.1	<0.05	99.3	<0.05
L. infantum	2	2.5 (-0.91, 5.9)	1.4	0.15	96.9	<0.05
L. braziliensis	2	8.5 (-4.3, 21.5)	1.2	0.19	89.9	0.002
Type of stilbenes						
RSV & its derivatives	6	29.09 (20.2, 37.9)	6.4	<0.05	99.1	<0.05
Stilbenes derivatives	8	5.1 (3.3, 6.8)	5.6	<0.05	96.6	<0.05

Discussion

Leishmaniasis is one of the health problems worldwide. To lower the risk of leishmaniasis, many herbal medicine are considered including polyphenols such as resveratrol. Previous published studies have reported the conflicting results regarding the leishmanicidal effects of RSV and other stilbenes in promastigote and amastigote stages of *Leishmania*. A total of nine studies were considered for promastigote and amastigote. A comprehensive review and meta-analysis of pertinent research found that *Leishmania* viability was dramatically reduced following treatment with RSV and other stilbenes in both promastigote and amastigote stages of *Leishmania*. In current study we did not observe not only the leishmanicidal effects of stilbenes types (RSV and other stilbenes) on different species of *Leishmania* but also the leishmanicidal effects on the different stages of the parasite (promastigote and amastigote stages).

The funnel plot, Begg's rank correlation, and Egger tests revealed that all papers included in this meta-analysis had a possible publication bias. Furthermore, there is a great deal of heterogeneity in this meta-analysis. Subgroup studies based on Leishmania species (*L. major*, *L. amazonesis*, *L. infantum*, and *L. braziliensis*) and stilbene types were undertaken to find the apparent heterogeneity (RSV and other stilbenes). Initially, we looked at several subgroups of Leishmania species and different types of stilbenes in each stage of Leishmania. The leishmanicidal effects of RSV and other stilbenes on the promastigote stage of *L. major*, *L. amazonesis*, and *L. infantum* were a lot more potent than they were before.

In contrast, the leishmanicidal effects of RSV and other stilbenes on the amastigote stage were substantial in the *L. major* and *L. amazonas* species. Furthermore, in both phases of Leishmania, subgroups of RSV and other stilbene derivatives significantly decreased Leishmania vitality. As a result, our findings show that RSV and other stilbene derivatives have leishmanicidal effects on promastigote and amastigote stages of Leishmania.

Resveratrol is a polyphenol found in nature that may have health advantages. According to a growing body of research, RSV has been shown to improve the pathogenic condition of metabolic disorders (30). Leishmaniasis can also be treated with resveratrol. Consequently, our findings in this meta-analysis showed that *Leishmania* viability decreased following resveratrol administration, which is consistent with the findings of several resveratrol-treated studies (23). *Leishmania* exist as morphologically distinct forms. Amastigotes are obligatory intracellular parasites of mononuclear phagocytes in mammals. Female sandflies have an extended motile promastigote morphology. The effects of resveratrol and its analogues on these two stages of *Leishmania* parasites were investigated in various studies. Promastigotes are external forms of *Leishmania* parasites, whereas amastigotes are intracellular forms. Resveratrol and its derivatives have been shown to have anti-leishmanial action in several investigations (31). Ferreira *et al.* (2014) reported that resveratrol has an anti-leishmanial activity against *L. amazonesis* in both the promastigote and amastigote stages (23). Coimbra *et al.* (2016), evaluating the leishmanicidal effects of resveratrol analogs showed that these compounds have different effects on *Leishmania* species (25). In this respect, Kedzierski *et al.* (2007) reported the effects of resveratrol and its hydroxylated analogs against *L. major*. The results demonstrated the leishmanicidal effects of resveratrol on promastigotes and intracellular amastigotes stages However, its hydroxylated analogs only showed anti-leishmanial activity against promastigotes (22). Furthermore, research on the anti-promastigote activity of trans-stilbenes and terphenyl compounds has revealed that one of the trans-stilbene derivatives has an anti-promastigote impact similar to pentostam, a critical anti-leishmaniasis medicine (16). Tolomeo et al. revealed the anti-promastigote and anti-amastigote activities of TTAS (Trans-3, 4′, 5-trimethoxy-3′ -amino-stilbene) as one of the stilbene derivatives (29). Similarly, the effects of four resveratrol analogues, including pterostilbene, piceatannol, polydatin, and oxy-resveratrol, were assessed against *L. amazonesis* in research. The results revealed that the piceatannol analogue might be a viable chemical in further investigations for leishmaniasis therapy (24). In this regard, our analysis results support the leishmanicidal effects of RSV and other derivatives of stilbenes on promastigote and amastigote stages of *Leishmania*.

The leishmanicidal actions of RSV and other stilbene derivatives on the promastigote and amastigote stages of the *Leishmania* parasite might be due to various mechanisms. such as, phospholipid-mediated apoptosis. This mechanism might be one of the methods via which the Leishmania death process is triggered. Phospholipids are the most common lipids found in eukaryotic cell membranes. Phosphatidylserine (PS) is a phospholipid with a low quantity in most biological membranes, and it is only found in the inner plasma membrane of eukaryotic cells in normal conditions. PS is also involved in Leishmania infectivity (32). A central point in the host-parasite interaction involves the adhesion to and invasion of host macrophages, by the metacyclic promastigotes and amastigotes. For invasion, promastigotes and amastigotes both require receptor-dependent phagocytosis. Furthermore, studies show that exposing PS to the parasite's cell surface simulates apoptosis and induces macrophages in the host organism to phagocytose the parasite (33). Investigations on amastigotes of *Leishmania amazonesis* showed that the signaling via exposed PS is a critical mechanism for *Leishmania* establishment in the mammalian host. The PS on the surface of amastigotes inhibits macrophage inflammatory activity. This lipid promotes parasite internalisation and causes an anti-inflammatory response by inhibiting macrophage NO activity, boosting IL-10 message, and TGF-1 secretion by interacting with macrophages (34). Infected mice were also treated with a PS-targeting monoclonal antibody, which reduced parasite burdens and lesion progression while improving DC activation and antigen presentation in vitro. As a result, like apoptotic cell clearance, the identification of PS exposed on the surface of amastigotes plays a role in down-modulating DC activities (35). PS may also play a significant role in how leishmaniasis progresses because it can change how Leishmania organisms and host cells interact with each other.

In a study that conducted on the *L. amazonesis,* the promastigotes were treated or not treated with piceatannol and incidental death measured using the expression of annexin-V. According to their findings, piceatannol considerably raised the expression level of annexin-V compared to untreated controls (24). Another investigation into *L. infantum* promastigotes found that treating them with 10µg/ml compound 3 (resveratrol derivate) for 48 hours caused PS externalization, suggesting that apoptosis is the cause of parasite death, even though this chemical had no apoptotic effects on macrophage cells (26). TTAS (Trans-3, 4′, 5-trimethoxy-3′-amino-stilbene) was found to be a new stilbene derivative that caused PS to be released in L. infantum, which meant apoptosis had been activated. TTAS showed an LD_{50} on normal CFu-GM of 17.7µg/ml more than 6 times higher than that showed on the *L. infantum* strain (36). Several recent investigations have found that stilbenes and their analogues change the cell cycle of several Leishmania parasite species, with an increase of parasites in the sub G0/G1 phase of the cell cycle (14). Apoptosis is indicated by the presence of cells in the sub-G0-G1 phase. Other research found that TTAS made Leishmania stop in the G2–M phase of the cell cycle and make the sub-G1 apoptotic peak rise when cell dies (29).

The mitochondrial-induced apoptosis process is the second mechanism mediated by stilbenes. Apoptosis in *Leishmania* and mammals is comparable and includes

internucleosomal DNA breakage, phosphatidylserine exposure on the plasma membrane's external surface, and loss of mitochondrial transmembrane potential (37). Since maintenance of mitochondrial transmembrane potential is essential for the survival of a single mitochondrion-parasite, studies on the effects of resveratrol on *Leishmania* spp. have evaluated the mitochondrial integrity of promastigotes testing the mitochondrial membrane potential. Previous research findings show a reduction in mitochondrial membrane potential in parasites treated with resveratrol or it's derivate in comparison with untreated parasite populations (24).

Our meta-analysis has several advantages. First, this meta-analysis comprehensively summarizes the evidence data on an anti-leishmanial activity resveratrol and their derivatives. Second, the included studies were conducted in different countries that are including the high prevalence of leishmaniasis. Third, our search strategy was very detailed and spanned multiple databases.

This systematic review and meta-analysis have several limitations: First, due to the small number of included studies for stilbenes and its derivatives, we could not fully understand the leishmanicidal effects of stilbenes and their derivatives. Second, despite using random-effect models to combine the pooled mean of IC50 across included research, there was significant heterogeneity between included studies, preventing an accurate evaluation. Third, the sample sources for eligible studies were countries that may differ in the prevalence of *Leishmania*. Finally, given these limitations, the findings should be interpreted with caution, because the scarcity of large studies limits the reliability of this meta-analysis.

Conclusion

In conclusion, data from several primary investigations demonstrated that *Leishmania* viability was significantly reduced in the promastigote and amastigote phases. However, further research is needed to evaluate and employ this compound as a potential leishmaniasis therapy in the future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethics Approval and consent to participate

The ethical approval was not necessary for the reason that our study was a meta-analysis belonging Availability of data and materials.

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